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MECHANISM OF SHOCK

EFFECTS OF INTRAVENOUS INJECTION OF SALT SOLUTION IN COLLAPSE INDUCED BY MECHANICAL IMPOUNDING OF BLOOD IN THE SPLANCHNIC REGION IN NORMAL AND IN HYPERTHYROID DOGS

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The fundamental pathologic process in both medical and surgical shock is the impounding of a large volume of stagnant or sluggishly flowing blood in dilated capillaries in some specific region or more diffusely throughout the body. This process results in a reduction in the volume of circulating blood, loss of fluid from the blood, increase in concentration of the blood and anoxemia of the tissues and organs in which the blood is impounded. Because of the shunting of a large percentage of the total volume of blood into the dilated vessels, the heart does not receive enough blood to maintain a normal systemic pressure. Attempts to increase the volume of the circulating blood by intravenous injection of fluids are usually without permanent effect. Since the mechanism of these processes is still but poorly understood, it seemed possible that information of value concerning some of the problems of shock might be obtained from experiments in which warm physiologic solution of sodium chloride was injected intravenously while a considerable part of the total volume of the blood was impounded in a known region by mechanical means, with only the necessary anesthesia as a complicating factor.

The literature dealing with shock [1] and with the exchange of fluids between blood and tissues [2] has been reviewed so extensively that it is deemed unnecessary to lengthen this paper by a repetition of such reviews.

From the Department of Pathology, Northwestern University Medical School.

1. Cannon, W. B.: Traumatic Shock, Philadelphia, D. Appleton & Co., 1923. Moon, V. H., and Kennedy, P. J.: Arch. Path. **14**:360, 1932. Rukstinat, G. J.: ibid. **14**:378, 1932.

2. Adolph, E. F.: Physiol. Rev. **13**:366, 1933. Gortner, R. A.: Am. Rev. Biochem. **1**:21, 1932; **3**:1, 1934.

EXPERIMENTAL PROCEDURE

In our experiments we employed the method of mechanically constricting the hepatic veins described by Simonds and Brandes.[3] This procedure induces an immediate fall in blood pressure of from 40 to 80 mm. of mercury, and the pressure continues at this level with little further fall or a slow gradual decrease for from five to thirty minutes. This almost constant level of pressure indicates that beyond a certain point blood does not continue to accumulate behind the mechanical barrier. During mechanical constriction of the hepatic veins the outflow from the cannulated thoracic duct is increased fivefold.[4] On release of the mechanical constriction, the blood pressure rises promptly to its original level, and the animal recovers.

This report is based on a study of fifteen dogs, nine of which were normal and six in a state of hyperthyroidism induced by feeding 10 Gm. of desiccated thyroid daily for from ten to fourteen days immediately preceding the experiments. That the latter six dogs were thyrotoxic was indicated by marked nervousness and hyperexcitability, by diarrhea and by loss of from 3.5 to 32.6 per cent of their original body weight during the period of thyroid feeding. Thyrotoxic dogs were used in these experiments because of the generally accepted belief that there is peripheral vasodilatation with increase in capillary permeability in hyperthyroidism. We were especially concerned with the rate of loss of fluid from the blood as indicated by fall in blood pressure and change in blood volume.

Under ether anesthesia, maintained at a nearly constant level by a mechanical device on the ether reservoir, cannulas were placed in the trachea and carotid artery, and the jugular and femoral veins were exposed. The cannula in the carotid artery was connected with a manometer for tracing change in blood pressure. The duration of these experiments ranged from fifteen minutes to more than an hour. As soon as the cannulas had been connected, the abdomen was opened and an 18 inch (45.7 cm.) rubber tube of the same size as that used on blood-counting pipets was placed about the hepatic veins in readiness for mechanical constriction. The mere placing of this ligature in position induces a fall in blood pressure. In the nine normal dogs this fall was of short duration and averaged 11 mm. of mecury, or 8.1 per cent of the initial pressure. In nine hyperthyroid dogs, including the six studied here, putting the ligature in position induced a greater and more lasting reduction in blood pressure, with an average fall of 37.4 mm. of mercury, or 33.1 per cent of the initial pressure.[5]

One minute after the hepatic veins were mechanically constricted, intravenous injection of warm physiologic solution of sodium chloride was begun. The quantity injected varied from 20 per cent to somewhat more than 100 per cent of the calculated initial blood volume of the animal used.

OBSERVATIONS

The blood pressure curves in these experiments exhibited consistent uniformity in general outlines, but there were significant differences in certain details between curves for normal and those for hyperthyroid dogs. In each group the blood pressure fell abruptly on constriction of the hepatic veins; it rose steadily during the injection of the salt solu-

3. Simonds, J. P., and Brandes, W. W.: Am. J. Physiol. **72**:201, 1925.

4. Simonds, J. P., and Brandes, W. W.: J. Immunol. **13**:11, 1927.

5. Hepler, O. E., and Simonds, J. P.: Proc. Soc. Exper. Biol. & Med. **34**: 534, 1936.

tion, declined invariably and uniformly after completion of the injection and rose abruptly when the hepatic veins were released (fig. 1). Actual changes in blood pressure in two normal and two hyperthyroid dogs are given in table 1.

Certain significant differences were manifest in the blood pressure curves of the normal and thyrotoxic animals. In the first place the

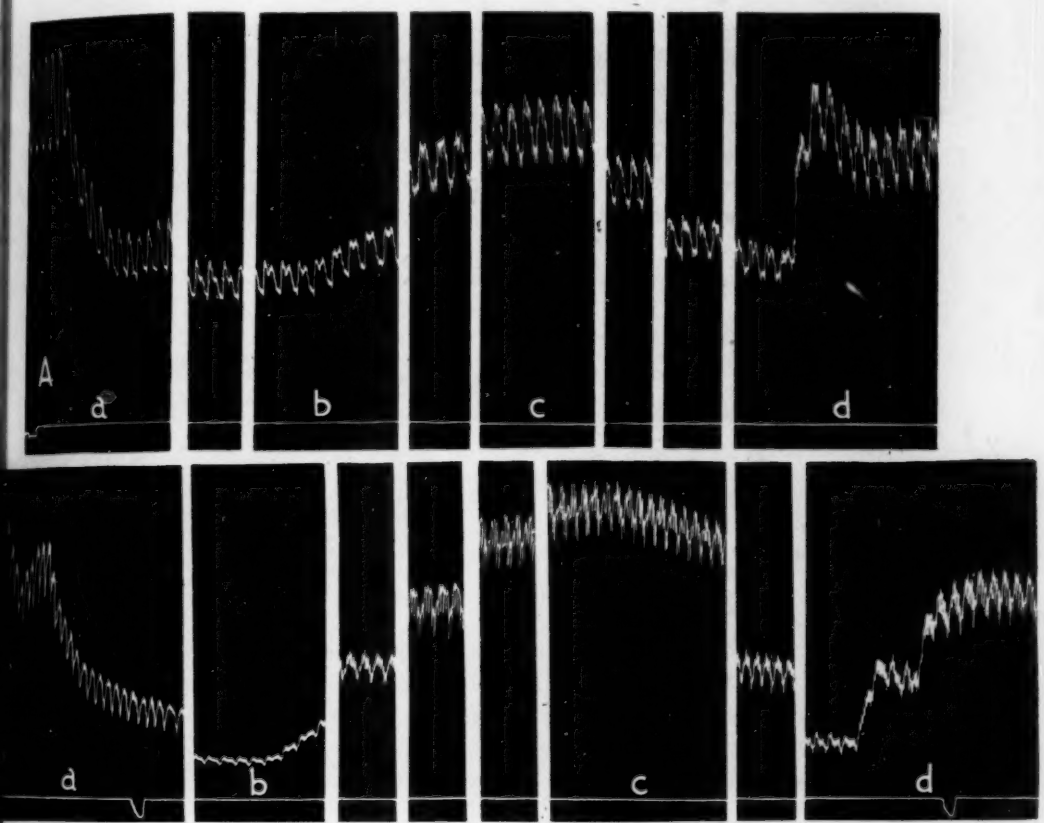

Fig. 1.—*A*, serial sections of a continuous tracing of the blood pressure from a normal dog that received intravenously warm salt solution equivalent to approximately 20 per cent of the calculated initial blood volume: (*a*) constriction of hepatic veins; (*b*) beginning of injection of salt solution; (*c*) maximal pressure induced by added salt solution; (*d*) release of hepatic veins. *B*, serial sections of a continuous tracing of the blood pressure of a hyperthyroid dog that received intravenously warm salt solution approximately equal to the calculated initial blood volume: *a*, *b*, *c* and *d* have the same significance as in *A*.

hyperthyroid dogs were more sensitive to the manipulation incident to putting the ligature in place, and their blood pressure immediately before constriction was applied to the hepatic veins was consistently lower than

that of the normal animals at the corresponding stage of the experiment. The blood pressure of normal dogs at the time mechanical constriction was applied to the hepatic veins ranged from 100 to 132, with an average of 115 mm. of mercury. The corresponding pressures in the hyperthyroid dogs ranged from 54 to 95, with an average of 73 mm. of mercury. The actual reduction in blood pressure induced by constriction of the hepatic veins was greater in the normal dogs than in the hyperthyroid animals, the average pressure being 40 and 29 mm. of mercury, respectively, or 34.8 and 39.7 per cent of the original pressure. The maximum blood pressure induced by injection of salt solution during mechanical constriction of the hepatic veins ranged in normal dogs from 60 to 126, with an average of 93 mm. of mercury, i. e., 81 per cent of the average original pressure. In the hyperthyroid dogs the corresponding range of pressure during injection of salt solution was from 46 to 80, with an

TABLE 1.—*Changes in Blood Pressure in Two Normal and Two Hyperthyroid Dogs*

	Normal Dogs		Hyperthyroid Dogs	
	No. 1	No. 16	No. 8	No. 13
Blood pressure before ligature was placed, mm. of mercury	116	115	132	105
Preconstriction blood pressure, mm. of mercury	116	100	80	88
Minimal blood pressure after constriction	68	20	28	42
Salt solution injected, percentage of initial blood volume	40	108	47	112
Maximal blood pressure from injection of salt solution	90	96	65	72
Minimal blood pressure after injection of salt solution	82	84	32	50
Blood pressure after release of constriction	112	114	54	72

average of 62 mm. of mercury, i. e., 85 per cent of the average preconstriction pressure. In from one to three minutes after the injection the pressure had declined in the normal group to an average of 55 mm. of mercury (47.8 per cent of the original level); in the thyrotoxic group, to 33 mm. of mercury (45.2 per cent). The most marked difference between the normal and hyperthyroid dogs followed release of the hepatic veins. In the normal group, release of constriction was followed by a rise to levels ranging from 96 to 122, with an average of 113 mm. of mercury, or only 2 mm. below, or 99 per cent of, the average pressure immediately preceding constriction. In the thyrotoxic dogs, release of the hepatic veins was followed by a rise to from 50 to 72, or an average of 57 mm. of mercury, 16 mm. below, or only 78 per cent of, the average pressure before constriction (table 2 and fig. 1 *B*).

From the foregoing analysis of our results, it is evident (1) that during mechanical constriction of the hepatic veins intravenous injection of physiologic solution of sodium chloride in amounts up to slightly more than the original estimated total volume of blood does not raise the

systemic pressure to its original level; (2) that within three minutes after completion of the injection the systemic pressure subsides to within 13 per cent of the constriction level in normal dogs and to within 5.5 per cent of that level in thyrotoxic dogs, and (3) that on release of the hepatic veins the blood pressure rises to within 99 per cent of its preconstriction level in normal dogs and to within only 78 per cent of that level in hyperthyroid dogs.

When blood pressure is reduced as a result of mechanically impounding blood in some region of the body, the degree of reduction in pressure is quantitatively related to the amount of blood so impounded and diverted from the general circulation. After impounding of blood by mechanical constriction of the hepatic veins, the systemic blood pressure falls to a fairly constant level equivalent to from 35 to 70 per cent of

TABLE 2.—*Changes in Blood Pressure in Six Normal and Six Hyperthyroid Dogs*

Average	Six Normal Dogs	Six Hyperthyroid Dogs
Blood pressure before constriction of hepatic veins, mm. of mercury	115	73
Minimal blood pressure after constriction of hepatic veins, mm. of mercury	40	29
Maximal blood pressure from injection of salt solution	93	62
Minimal blood pressure after injection of salt solution	55	33
Blood pressure after release of hepatic veins	113	57
Blood pressure after constriction of hepatic veins, percentage of original pressure	34.8	39.7
Blood pressure from injection of salt solution, percentage of original pressure	81.0	85.0
Blood pressure after injection of salt solution, percentage of original pressure	47.8	45.2
Blood pressure after release of hepatic veins, percentage of original pressure	99.0	78.0

the original pressure. This wide range is due in part to the impossibility of completely occluding the hepatic veins in some animals because of differences in the anatomic arrangement of these vessels. In every case, however, a great volume of blood was impounded behind the barrier. The fact that in many experiments of this type the level of the blood pressure during constriction remained fairly constant for the animal used indicates that the impounding of blood behind such a barrier did not continue indefinitely but that the impounded blood reached some sort of equilibrium with the blood still in the systemic circulation. Intravenous injection of salt solution did not appear to alter this equilibrium materially. For in these experiments no amount of salt solution given intravenously maintained the systemic pressure much above the constant constriction level for more than three minutes. Hence it appears that when physiologic solution of sodium chloride is injected into a systemic vein during constriction of the hepatic veins the increased volume of

diluted blood in the reduced systemic circulation again accumulates behind the mechanical barrier and escapes into the viscera drained by those veins.

It is possible, therefore, that in shock there may be some relationship between the size, the location or the nature of the reservoir in which the blood is impounded and the level at which the systemic pressure can be maintained. The continued fall of blood pressure in medical and surgical shock may be due (1) to an extension of the region in which blood is impounded or (2) to a further dilatation of capillaries within the limits of the original region or (3) to increased loss of fluid from the blood through capillary walls. The latter two factors appear to be the most important under the conditions of our experiments.

It is also evident from the analysis of our results, (1) that the thyrotoxic state in the dog is accompanied by a loss of stability of the vasomotor mechanism and (2) that the experiments were complicated by some factor in the thyrotoxic animals which did not exist in the normal dogs.

The total volume of blood in each of these animals was estimated as equivalent in cubic centimeters to 9.5 per cent of the body weight in grams. Changes in blood volume resulting from injection of salt solution were determined by comparing the number of red cells per cubic millimeter of blood, the number of grams of hemoglobin per hundred cubic centimeters and the hematocrit determinations before injection with similar determinations one minute after injection and one minute after release of the hepatic veins. This method is admittedly subject to gross errors. The more accurate dye and gas methods were not applicable under the conditions of our experiments. Attempts to determine even relative changes in blood volume by the methods which we used are, under the conditions of these experiments, hampered by at least three uncontrollable factors: 1. During the constriction of the hepatic veins we were injecting fluid into a greatly reduced vascular bed. 2. The degree of constriction of the hepatic veins could not be made constant for all animals. 3. In the thyrotoxic dogs some unknown, probably vasomotor, factor affected the results. However, we were not interested so much in actual blood volumes as in finding some indication of the rate of loss of fluid from the blood. The red cell counts and hemoglobin determinations furnished information on this point.

For the purpose of presenting the results of these experiments it was convenient to consider the calculated initial blood volume in each dog as 100 and to reduce all other computations for each animal to a comparative basis. The actual figures thus obtained are shown in table 3. From this table certain facts are evident: 1. The blood volume after injection of salt solution, computed from red cell counts and hemoglobin

determinations, during constriction of the hepatic veins may be greater or less than the calculated initial blood volume plus the injected salt solution. This is true for both groups of animals. Omitting from the calculation the animals for which the figures are questionable, the average for each group is slightly higher (179 and 162 for the normal ones, and 179 and 172 for the thyrotoxic dogs). This indicates that during constriction a considerable amount of salt solution escaped from the circulation within one minute after completion of the injection, which

TABLE 3.—*Blood Volumes Calculated During Course of Experiments*

Dog*	Calculated Initial Blood Volume	Calculated Blood Volume Plus Salt Solution	Blood Volume Computed from Red Blood Cell Count, Hemoglobin Content and Hematocrit Reading		Calculated Blood Volume Plus Second Salt Solution	Blood Volume Computed from Red Blood Cell Count, Hemoglobin Content and Hematocrit Reading	
			During Constriction	After Release		During Constriction	After Release
N 4	100	116	116	109			
N 2	100	141	760 ?†	136			
N 3	100	105	168	...	139	510 ?	109
N 5	100	135	...	116			
N 1	100	140	115	118	173	128	
N 10	100	151	162	117	181	142	123
N 12	100	197	141	113			
N 11	100	214	222	136	264	259	130
N 16	100	237	334	144			
Average.....		162	179	122			
T 8	100	146	161	134			
T 9	100	156	137	120			
T 7	100	159	228	129			
T 15	100	213	141	125			
T 14	100	222	273	152	252	163	
T 13	100	184	121	...	236	150	130
Average.....		172	179	132			

* N designates normal dogs; T, thyrotoxic dogs.
† The question marks after the figures in columns 4 and 7 indicate that the differences between the calculations based on red cell counts and hemoglobin determinations were of sufficient magnitude (i. e., greater than 0.5) to render the figures of doubtful value.

lasted from two to twelve minutes depending on the quantity injected. 2. The loss of fluid from the blood continues after release of constriction of the hepatic veins. The escape of fluid was so rapid that in those dogs which received large quantities of salt solution a liter or more of fluid left the circulation within from ten to fifteen minutes. But in no animal did the computed blood volume return to the calculated initial volume during the period of the experiment.

The facts just cited furnish evidence (1) of the rapidity with which an excess of fluid escapes from the circulating blood and (2) of the capacity of the tissues to store fluids temporarily in an emergency. In our experiments factors in the escape of fluid from the blood were

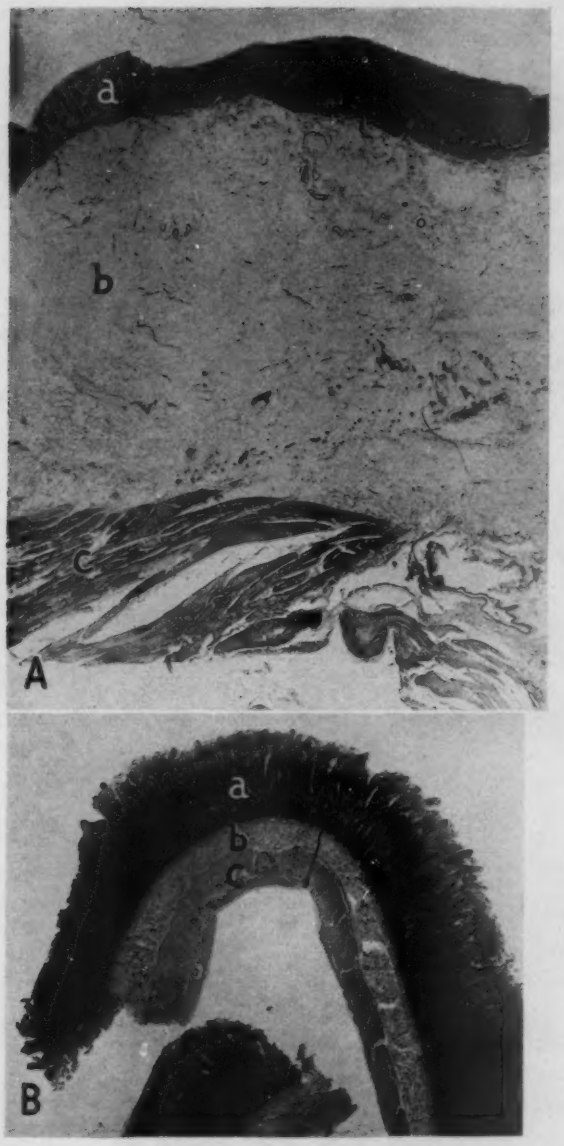

Fig. 2.—Edema of the submucosa of the stomach (*A*) and small intestine (*B*) of dog 14: (*a*) mucosa; (*b*) submucosa; (*c*) muscular coats.

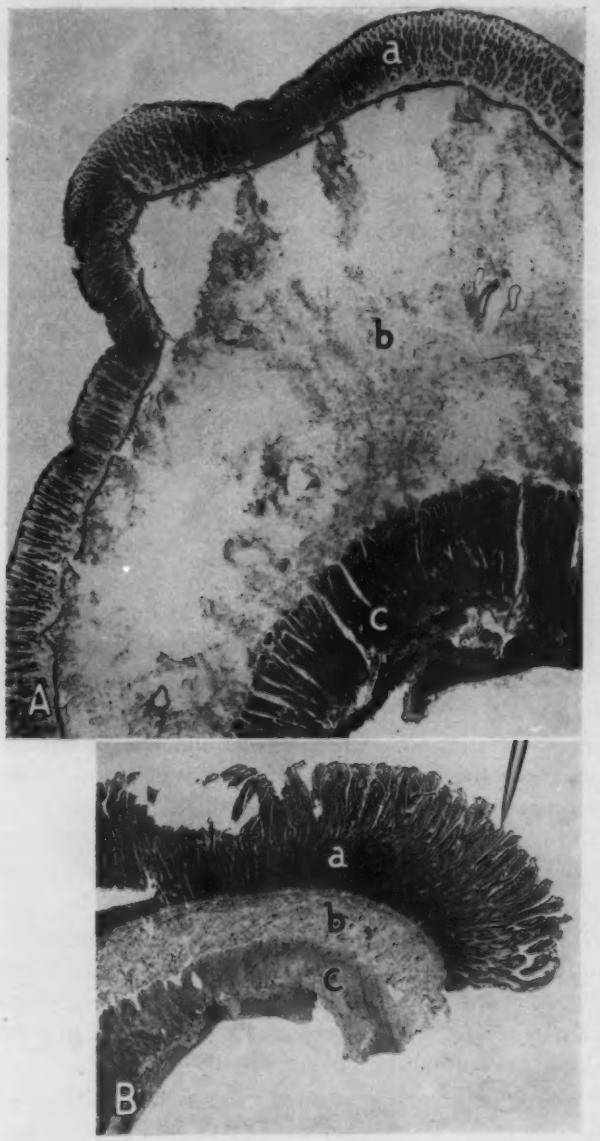

Fig. 3.—Edema of the submucosa of the stomach (*A*) and intestine (*B*) of dog 16: (*a*) mucosa; (*b*) submucosa; (*c*) muscular coats.

increased capillary pressure in the region of impoundment behind the mechanical barrier and reduction in the osmotic pressure of the plasma proteins as a result of dilution by the injected salt solution. Davis [6] called attention to these factors as operative when Locke's solution is injected intravenously. The use of such fluids is thus definitely contraindicated in shock.

The places of escape of fluid from the vessels were traced by gross and microscopic examination of organs and tissues. In no animal was the amount of fluid in the peritoneal cavity significantly large. The retroperitoneal tissues and the walls of the gallbladder and small intestine were moderately edematous. The liver weight-body weight ratio and the microscopic appearance of the liver did not indicate accumulation of any great amount of fluid in that organ. The bladder did not contain an excess of urine. The stomach was a conspicuous avenue of escape for the injected fluid. In animals which received large amounts of salt solution, the lumen of the stomach contained amounts of almost clear fluid up to 200 cc., and the submucosa of the fundus was distended with fluid and measured up to 8 mm. in thickness. The extent of this edema is shown in figures 2 and 3.

SUMMARY

When blood is impounded in the liver and gastro-intestinal tract by mechanical constriction of the hepatic veins in dogs the systemic blood pressure falls abruptly to a level equivalent to from 35 to 70 per cent of the original pressure, and this is maintained with remarkable constancy for periods of at least thirty minutes.

Injection of physiologic solution of sodium chloride during the period of constriction of the hepatic veins in quantities up to somewhat more than the estimated initial blood volume induces a rise in systemic blood pressure during the injection, but the pressure falls almost to the previous low level within from one to three minutes after the injection is stopped.

The blood volume, computed from the red cell count and from hemoglobin and hematocrit determinations, after injection of salt solution and while the hepatic veins are still constricted may be either greater or less than the calculated initial volume plus the salt solution injected, the average in each group of dogs being slightly greater.

Blood volumes similarly computed after release of the hepatic veins were invariably much lower than the calculated initial volumes plus the salt solution injected.

The rate of fall in blood pressure and reduction in blood volume after injection of salt solution under the conditions of these experiments

6. Davis, H. A.: Proc. Soc. Exper. Biol. & Med. **33**:245, 1935.

indicate the rapidity with which fluid escapes from the circulating blood when a considerable part of the total volume of blood is impounded in a known region of the body.

Thyrotoxicosis in dogs adds a complicating factor in experiments of this type.

In those dogs which were submitted to injection of large amounts of salt solution a surprising amount of fluid was found to have escaped into the lumen of the stomach and into the gastric submucosa. The peritoneal cavity contained relatively little fluid, and the retroperitoneal tissues and the walls of the gallbladder and small intestine were moderately edematous.

SPONTANEOUS AND EXPERIMENTAL AMEBIC INFECTION IN REPTILES

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The causes, nature and incidence of diseases among Reptilia are generally unknown. However, representative groups of this phylum are constantly on display at the zoological garden in Philadelphia, and, according to the autopsy records, many deaths have been due to intestinal disorders. In some instances these conditions could be attributed to bacteria or to inadequate food, but often the etiology was obscure. In 1933 we reported that one form of enteritis of certain species of reptiles may be caused by endamebas.[1]

Although amebic infection in reptiles is now known to have occurred at the zoological garden in Philadelphia as early as 1922, the first recognized case was that of a monitor lizard (Varanus varius) which died on Oct. 3, 1931, after fifteen months' exhibition. Prior to this discovery, only rare or unusual reptiles were examined post mortem, but thereafter, autopsy of all species and microscopic examination of the intestinal contents became the routine practice.

As additional reptiles with amebiasis became available for study, the morbid processes seemed to be fairly specific and to have features in common with amebiasis of higher vertebrates. Hence we initiated a study of the endameba and the disease with which it is associated. A full account of the first part of this investigation has been presented;[2] that of the latter half is given now.

We first called attention to this disease and gave a description of the parasite in 1933 and 1934.[3] Later, in 1934, successful experimental

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From the Penrose Research Laboratory of the Zoological Society of Philadelphia, the Department of Pathology of the University of Pennsylvania and the Department of Comparative Pathology of Harvard University Medical School and School of Public Health.

1. Ratcliffe, H. L., and Geiman, Q. M.: J. Parasitol. **20**:139, 1933.
2. Geiman, Q. M., and Ratcliffe, H. L.: Parasitology **28**:208, 1936.
3. Ratcliffe and Geiman.[1] Ratcliffe, H. L., and Geiman, Q. M.: Science **79**:324, 1934.

infections were reported.[4] These preliminary papers were followed by a brief report from Rodhain [5] that amebiasis had been found in reptiles at the zoological garden in Antwerp and that the parasite was a new species, Endamoeba invadens. More recently, Rodhain and van Hoof [6] obtained experimental infections and discovered additional cases of the spontaneous disease. Data offered in support of the opinions that the ameba which causes amebiasis in reptiles is a new species and that the name Endamoeba invadens should be accepted for the present were discussed in our recent paper.[2]

MATERIAL AND METHODS

The tissues used in this study were taken from eighty-one animals, fifty-four of which had been experimentally inoculated. The original plan included killing half of the experimental animals at regular intervals after inoculation, but the rate of development of the disease and the extent of the lesions varied so much that this had to be abandoned.

In order that the pathologic changes in amebic infection might be compared with those in other forms of intestinal disease of reptiles, tissues were collected from twenty-five specimens (seventeen colubrids, five crotalids and three iguanids) that died of acute enteritis in which amebas were not involved.

For histologic examination, tissues were fixed in Zenker's fluid, embedded in paraffin and, for routine purposes, stained with Mayer's hemalum and eosin. Heidenhain's iron-alum-hematoxylin was used for study of amebas in the tissues, and many duplicate sections were stained with eosin-methylene blue to demonstrate bacteria.

Two strains of E. invadens, A and B (Geiman and Ratcliffe, 1936), were used for experimental studies. Both grew vigorously on the gastric mucin-saline-rice starch medium,[7] and produced large numbers of cysts. Inocula were obtained from cultures that had been allowed four or five days' growth at room temperature and from seven to ten days' storage at from 5 to 7 C. for maturation of cysts.

Water snakes (Natrix rhombifera, Natrix sipedon and Natrix cyclopion), anolian lizards (Anolis carolinensis), small alligators (Alligator mississippiensis) and young kittens were inoculated. The mammals were used because of the close morphologic similarity of E. invadens and Endamoeba histolytica. Alligators

4. Ratcliffe, H. L., and Geiman, Q. M.: J. Parasitol. **20**:328, 1934.

5. Rodhain, J.: Compt. rend. Soc. de biol. **117**:1195, 1934.

6. Rodhain, J., and van Hoof, M. T.: Compt. rend. Soc. de biol. **117**:1199, 1934; **118**:1646, 1935.

7. The original account of the preparation of this medium omitted mention of the quality of salt to be used. The formula is: gastric mucin, 0.3 Gm., "ground alum" salt, 0.5 Gm.; water, 100 cc. About 2 mg. of sterile rice starch is added to each culture tube at the time of seeding the tubes. Highly refined sodium chloride of several makes was not as satisfactory as "ground alum" salt, a recrystallized rock salt used in meat packing. This product contains approximately 99 per cent sodium chloride, the remainder being made up of calcium, potassium and magnesium as chlorides and phosphates.

and anolian lizards were the only readily available species of the general type of reptile. Water snakes made up the greater number of experimental animals because they were easily obtained. The specimens of Natrix rhombifera were collected near Harper, Kan., those of Natrix sipedon from the vicinity of Philadelphia and those of Natrix cyclopion from the vicinity of Silver Springs, Fla.

Complete fecal examination of the reptiles before experimental inoculation was impossible. Either fecal material could not be obtained, or it was mixed with so much urinary excrement that microscopic study was impracticable. However, the animals had been shipped from the field in small groups. These groups were kept intact, and when animals were needed for inoculation half of each group were killed for control examinations. Stock animals that died were also examined as controls.

In order to prevent the introduction of foreign endozoic protozoa, the snakes were fed canned fish, and the lizards and alligators received chopped meat.

Each of the snakes and alligators that was inoculated received approximately the quantity of cysts which was obtained from 5 cc. of medium in a test tube 12 mm. in diameter. The anolids were each given about 0.2 cc. of sediment from a culture. Heavy suspensions of cysts and trophozoites were mixed with milk and fed to the kittens.

One group of water snakes (Natrix rhombifera) were inoculated with suspensions of bacteria from the amebic cultures.

SPONTANEOUS INFECTIONS

Case Histories.—It has been impossible to trace completely to its source any animal that died of spontaneous amebic infection, but the available information is given in this section. Table 1 shows the date of death of each animal and the distribution of the lesions. If cases occurred as groups, they are tabulated accordingly.

Cases 1 to 5 were unrelated either by contact or by articles of food in which E. invadens might have been carried. The food for the Australian lizards, Varanus varius and Tiliqua scincoides, was meat and eggs; the snakes, Pseudoboa cleclia and Natrix sipedon, received frogs and fish, and the king snake, Lampropeltis getulus, was fed on mice. Cases 21 and 22 also appear to have been isolated instances of the disease. The specimen of Varanus salvator was exhibited alone and was fed meat and eggs. Two specimens of Varanus komodoensis were exhibited together in the collection of the zoological society of New York. These animals died within a few days of each other, but only one was infected.

Case 23, in Cyclagras gigas, has the following history: The animal was received with two others of this species by a local dealer in a shipment of reptiles from Amsterdam and died shortly after arrival. In the meantime, the other two animals were sold to zoological gardens in the United States, where they lived for only a few days, and their bodies were destroyed without examination.

About a year later, this dealer received a king snake (Lampropeltis getulus) from a collector in Florida, and it died on the day of arrival (case 24). This animal was said to have been captured about ten days before death and after capture had not been in contact with other reptiles.

Cases 25 to 27 occurred in boas that had been purchased from the dealer who sent in the animals in cases 23 and 24. The South American boa (Constrictor constrictor) was received on May 29, 1935. It died on August 17, following

The Cuban boas (Epicrates angulifer) were placed on exhibition with the South American boa on August 1. They refused food and died thirty-one days later.

In cases 6 to 12 and 13 to 20, amebiasis developed among two groups of water snakes, Natrix rhombifera and Natrix sipedon. These animals were purchased in April 1933, having been shipped to Philadelphia shortly after their capture. Originally twenty-four of each species were exhibited in adjoining indoor cages, in which the combined floor space was about 20 square feet (1.85 square meters). During the four months following receipt, animals from each group died but were not infected. Then the disease appeared in the group of Natrix rhombifera, seven animals dying in thirty-three days. Nineteen days later the first case developed in the adjacent group of Natrix sipedon, eight of these dying

TABLE 1.—*Reptiles in Which Spontaneous Infection by Endamoeba Invadens Has Been Found at the Zoological Garden in Philadelphia*

Case	Animal	Date of Death	Gross Lesions*			
			Stomach	Small Intestine	Colon	Liver
1	Varanus varius	10/ 3/31	—	+	+	+
2	Tiliqua scincoides	9/22/32	—	—	+	—
3	Pseudoboa clelia	10/ 3/32	—	—	+	+
4	Natrix sipedon	1/20/33	—	+	+	—
5	Lampropeltis getulus	2/20/33	—	+	+	—
6 to 9	Natrix rhombifera	8/2/33 to 8/16/33	1	1	4	3
10	Natrix rhombifera	8/25/33	+	—	—	—
11, 12	Natrix rhombifera	9/3/33, 9/4/33	0	1	2	2
13 to 20	Natrix sipedon	10/28/33 to 12/13/33	8	5	8	8
21	Varanus salvator	2/19/34	—	—	+	+
22	Varanus komodoensis†	.....	—	—	+	+
23	Cyclagras gigas†	.....	+	+	+	+
24	Lampropeltis getulus†	.....	—	—	+	+
25	Constrictor constrictor	8/17/35	—	+	+	+
26	Epicrates angulifer	9/ 2/35	—	—	+	+
27	Epicrates angulifer	9/ 2/35	—	—	+	+
28	Natrix sipedon pictiventris	9/15/36	+	+	+	+
29, 30	Natrix taxispilota	9/17/36	2	2	2	2
31	Natrix sipedon (killed)	9/18/36	+	+	+	+
32	Thamnophis sirtalis	11/ 9/36	+	+	+	+

* A minus sign means absence of disease; a plus sign, active disease; a plus minus sign, lesion of uncertin nature but associated with amebic infection.

† These animals were not the property of the Zoological Society of Philadelphia.

within fifty days. During the periods in which these deaths occurred, one specimen of Natrix rhombifera and three of Natrix sipedon died of other causes. Three specimens of Natrix sipedon remained after the disease subsided, but they were not infected.

The other spontaneous infections in this series developed among reptiles which were confined in a large outdoor enclosure of about 1,000 square feet (92.9 square meters). This space is well planted with low-growing shrubs, which cover rockwork and soil and provide ample dens for the animals. A stream runs through the enclosure forming shallow pools. Half of the floor space is usually dry; the remainder is moist. For three years a collection of turtles, lizards and colubrids had been kept in this enclosure. Many species reproduced; losses were definitely lower than among some reptiles of the same species that were kept within doors, and amebiasis had not developed among them.

From April 20 to May 20, 1936, thirty-five specimens of Natrix sipedon, thirty-five of Natrix taxispilota and thirty-five of Natrix sipedon pictiventris, all

newly captured animals, were released in this enclosure in association with the other reptiles mentioned as regular inhabitants. Small marine fish, "smelts," were offered as food, but no effort was made to see that the snakes took food regularly. These animals (Natrix) remained in relatively normal condition for about three months. Two of them died ninety-six days after arrival, and within the following twenty days sixty others died or were killed as unfit for exhibition. None was infected with E. invadens. On September 15, however, one of Natrix sipedon pictiventris (case 28) died of amebiasis, and two days later two of Natrix taxispilota (cases 29 and 30) succumbed to the disease. On the day following, thirty others were killed, one of which (Natrix sipedon, case 31) presented typical lesions of amebic infection. The other specimens of Natrix were hidden under the rockwork and could not be examined at this time. Later, however, on November 19, a garter snake (Thamnophis sirtalis, case 32) died in this enclosure of amebiasis, and during a few warm days which followed the remaining members of Natrix in this enclosure were caught for examination, but none was infected.

Such autopsy records and tissue specimens as are available from reptiles that died before this study began show that amebiasis developed in some of these animals also. Two (Varanus salvator), examined on July 31, 1922, and on June 20, 1927, presented disease changes of the ileum and colon which were typical of amebiasis, and in sections of the colon of one animal amebas indistinguishable from E. invadens were found. Similar pathologic changes developed also in the lower intestine of three specimens of Varanus varius, which died on October 5, 15 and 17, 1922. Again, sections of the colon of one animal contained amebas. Tissues of the other animals were not sectioned, and other information concerning them is lacking.

EXPERIMENTAL INFECTIONS

Strain A.—Experiment 1. Ten months after the isolation of this strain of E. invadens in culture from a water snake (Natrix rhombifera, case 9), eleven specimens of Natrix rhombifera, five of Natrix cyclopion and six of Natrix sipedon were inoculated with mature cysts. The first of these animals died of the infection twenty days after inoculation, but, when one of Natrix rhombifera was killed two days later, the disease changes consisted of a few small ulcers of the mucosa of the colon, and the other organs were normal. Hence no other animal of this group was killed until thirty days after inoculation. In one of these (Natrix cyclopion) lesions were limited to the colon. In three others (Natrix sipedon) atypical lesions had developed in the colon, while the liver had undergone characteristic changes. One (Natrix rhombifera) killed at thirty-four days had atypical disease in the liver, while the intestines were normal, and another of this species, killed at forty-four days, was not infected, although amebas had been recovered from its feces seven days after inoculation. One other animal, no. 34, did not contract amebiasis, its death twenty days after inoculation being due to perforation of the stomach by a nematode, Ophidascaris sp. Amebas were recovered from the large intestine.

Table 2 shows the interval between inoculation and death and the distribution of lesions for each animal. Fourteen of the twenty-two animals died of the disease between twenty and thirty days after they had received the inocula, the average period between inoculation and death being twenty-three days.

Experiment 2. When strain A had been under cultivation for about twenty-two months, twenty-seven specimens of Natrix rhombifera were inoculated with mature cysts. Growth of this strain of the endameba had become less vigorous as its age in culture increased, fewer cysts being formed than during the earlier experiments, so that each animal of this group received approximately half the

TABLE 2.—*Results of Inoculation of Three Species of Natrix with Strain A of Endamoeba Invadens When the Organism Had Been in Culture for Ten Months*

	Animal	Survival Time, Days	How Terminated	Gross Lesions*			
				Stomach	Small Intestine	Colon	Liver
Natrix rhombifera	1	20	Died	—	—	+	+
	2	22	Killed	—	—	+	—
	3	20	Died	—	—	+	+
	4	44	Killed	—	—	—	—
	5	26	Died	+	+	+	+
	23	21	Died	—	—	+	+
	24	22	Died	—	—	+	+
	25	22	Died	+	—	+	+
	26	23	Died	+	—	+	+
	27	25	Died	—	—	+	+
	28	34	Killed	—	—	±	+
Natrix cyclopion	29	20	Died	—	—	+	+
	30	23	Died	—	—	+	+
	31	30	Died	—	—	+	+
	32	30	Died	—	—	+	+
	33	30	Killed	—	—	+	—
Natrix sipedon	34	20	Died	—	—	—	—
	35	20	Died	—	—	+	+
	36	30	Died	—	—	+	+
	37	30	Killed	—	—	±	+
	38	30	Killed	—	—	±	+
	39	30	Killed	—	—	±	+

* A minus sign means absence of disease; a plus sign, active disease; a plus-minus sign, lesions of uncertain nature but associated with amebic infection.

TABLE 3.—*Results of Inoculation of Natrix Rhombifera with Strain B of Endamoeba Invadens When the Organism Had Been in Culture for Four Months*

Animal	Survival Time, Days	How Terminated	Gross Lesions*			
			Stomach	Small Intestine	Colon	Liver
9	20	Killed	—	—	+	+
10	20	Killed	± (micro.)	—	—	±
11	20	Killed	—	—	—	±
12	20	Killed	—	—	+	+
13	13	Died	+	+	+	+

* A minus sign means absence of disease; a plus sign, active disease; a plus-minus sign, lesions of uncertain nature but associated with amebic infection.

inoculum that had been given to each of the others. Twenty-five of the group died of amebiasis, their deaths occurring within from twenty-two to seventy-seven days after inoculation, with an average survival time of thirty-seven days. Two animals were killed, one thirty-one days after inoculation and the second at eighty-seven days. All of the snakes in this group acquired amebiasis, lesions occurring in the colon in twenty-three animals, in the liver in all, in the stomach

in fifteen and in the small intestine in four. Four of the group, including the two that were killed, presented advanced disease of the stomach and liver, but the small and large bowel were uninvolved, although amebas were recovered from the lumens.

Strain A1.—During experiment 1, amebas (strain A1) were isolated from animal 25 (table 2). Ten months after the isolation of this strain and twenty-two months after the original culture was obtained, eleven specimens of Natrix rhombifera were inoculated with mature cysts from cultures, each animal receiving approximately the same number of cysts as those listed in table 2. Ten snakes of this group died of amebiasis between twenty and thirty-four days after they received the inocula, the average time between inoculation and death being twenty-eight days. The other animal, killed thirty-eight days after inoculation, had typical changes in the colon and liver. Lesions developed in the colon and liver of every animal, and in the stomachs of two of them, but the small intestine was not involved in any animal.

Strain B.—Experiment 1. About four months after isolation of these organisms from Varanus salvator, five specimens of Natrix rhombifera were inoculated with mature cysts, the inocula being approximately equal to those in the first experiment with strain A.

One snake died thirteen days after inoculation and was found to have disease of the stomach, intestine, colon and liver. The others were killed seven days later and presented the following changes (table 3): Two animals had typical lesions of the colon and liver, while in the final ones the colon showed no amebas or lesions, but scattered areas of necrosis were found in the liver. The stomachs of these four snakes were normal to gross inspection, but a subcutaneous nodule in no. 10 was made up of inflammatory cells, among which were found amebas.

Experiment 2. About ten months after the first experiment with strain B, twenty anolian lizards (Anolis carolinensis) and seven small alligators (Alligator mississippiensis) were inoculated, receiving doses of mature cysts, which, in proportion to body weight, approximately equaled those for Natrix. The lizards died between four and twenty-four days after inoculation, but without signs of amebiasis, nor could E. invadens be recovered from their bodies. One alligator died at twenty days; the others were killed twenty-two days later, but the organism apparently did not colonize the intestines.

Experiment 3. Five kittens, litter mates about 5 weeks old, received large doses of mature cysts of strain B. They remained healthy, and repeated fecal examination failed to reveal the organisms.

COURSE OF DISEASE

Spontaneous Infections.—Animals in which spontaneous amebiasis developed did not present definite signs of disease. Many failed to take food for considerable periods before death, but in captivity reptiles may refuse food for from two to three months and remain healthy. Some specimens were poorly nourished when they died, but it was impossible to correlate the extent and severity of the disease with the state of nutrition.

Experimental Infections.—The water snakes refused food for two or more weeks after inoculation with cysts of E. invadens, and only

occasional ones could be induced to feed after this period. Possibly food other than canned fish might have been more attractive.

As the interval between infection and death increased, animals lost weight, but the extent and severity of the disease was not related to the state of nutrition.

Since the infected animals usually refused food, their intestinal discharges were composed chiefly of urinary excrement. Occasionally, however, during the early stages of the disease, blood-stained mucus was passed. This material contained cysts and trophozoites of E. invadens.

The survival time of snakes inoculated with E. invadens and dying of amebiasis varied from thirteen to seventy-seven days, and one which was killed eighty-seven days after infection had active disease in the stomach and liver. Survival periods varied most, from twenty-two to seventy-seven days, in the group of snakes for which the inoculum contained about half the number of cysts received by animals of other groups. Since clinical signs of infection were lacking, development of disease could not be followed and the organism may have remained in the lumen of the intestine for variable periods without invading the tissue. Once disease was initiated, it may have led to death in essentially the same time in all of the animals. In many cases the intestinal wall probably was attacked soon after inoculation, but in the only snake that apparently died of intercurrent disease amebas were recovered from the intact colon twenty days after introduction. In four animals, two of which died of amebiasis, pathologic changes due to amebas were limited to the stomach and liver, although the parasites were found in the small intestine and colon.

These cases of "atypical" amebiasis, as well as those in which "atypical" lesions occurred (table 2), are impossible of explanation at present. Perhaps in them partial recovery had taken place.

DISTRIBUTION OF LESIONS

Pathologic changes which have been associated with infection by E. invadens developed only in the stomach, small intestine, large intestine and liver. Amebas were sometimes found in the blood vessels of other organs, such as the lung, spleen and pancreas, but in these sites tissues were not affected to an appreciable extent. As compared with amebiasis of mammals, which is principally a disease of the large bowel, with hepatic abscess a relatively rare secondary complication, and involvement of the small intestine limited to the terminal part of the ileum, this disease of reptiles presents considerable differences in the distribution of lesions.

Spontaneous Infections.—In table 1 the presence or absence of lesions in the various segments of the intestinal tract and liver is indi-

cated. In most of the animals E. invadens apparently became established in the lumen of the colon and after initiating disease usually invaded the liver and often spread to the stomach and small intestine. The colon was involved in all except one animal (case 10, Natrix rhombifera), and in this animal disease was limited entirely to the gastric mucosa.

Hepatic disease developed in twenty-eight of the thirty-two animals, one or more specimens of each genus in the series, except Tiliqua scincoides (case 2), having involvement of the liver. Lesions of the mucosa of the small intestine were relatively common, seventeen animals belonging to six of the nine genera in the group having this segment of the intestine involved. Disease of the stomach was less frequent and developed only in specimens of Natrix rhombifera, Natrix sipedon, Cyclagras gigas and Thamnophis sirtalis.

Experimental Infections.—All types of lesions which were found in the group of animals dying of spontaneous amebiasis developed

TABLE 4.—*Distribution of Gross Lesions in Reptiles (Natrix) Dying After Spontaneous and Experimental Infection with Endamoeba Invadens*

Cases	Source of Amebas	Time Under Cultivation, Months	Average Survival Time of Snakes, Days	Gross Lesions			
				Stomach	Intestine	Colon	Liver
20	Natural	..	Unknown	14	12	19	17
14	Strain A	10	23	6	1	14	13
10	Strain A1	10	28	2	0	10	10
25	Strain A	22	36	15	4	23	25

also in the inoculated animals. However, disease of the stomach and small intestine was less frequent than among specimens of Natrix which died of natural infections, but it occurred more often as the interval between inoculation and death increased. The average survival time of all experimental animals in which ulcers of the stomach developed was forty days, and was fifty days in the fifteen cases from the last group in table 4. The snakes of this group received smaller inocula than other experimental animals.

Table 4 shows the average distribution of lesions in specimens of Natrix which died of spontaneous infection as compared with that in three groups of Natrix, all of which died after inoculation with strain A and strain A1 of E. invadens.

GROSS PATHOLOGIC CHANGES

With few exceptions pathologic processes that accompanied infection by E. invadens apparently developed first in the large intestine. Following the formation of lesions in the lower bowel, the amebas invaded the liver by way of the blood stream. In many instances the infection extended along the mucosa from the colon to the adjoining ileum, since

a distinct boundary (ileocecal valve) is absent in many animals in which the disease occurred (fig. 1). Ulceration of the mucosa of the stomach usually was secondary to disease in the large intestine, but in five snakes (Natrix) intestinal lesions were limited to the stomach and in four of these were accompanied by involvement of the liver. The following descriptions apply to all types of animals except when otherwise indicated and are based on material from both spontaneous and experimental cases.

Large Intestine.—The initial stages in the development of amebiasis of the large intestine were represented by discrete ulcers, from 1 to 5 mm. in width and irregular in outline, located just proximal to the valvelike constriction between the colon and the rectum (fig. 1). These

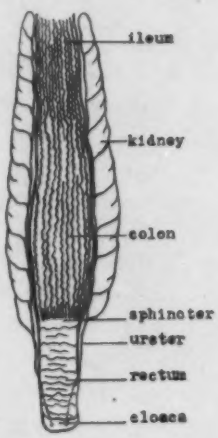

Fig. 1.—Diagrammatic sketch of the lower bowel of a colubrid. The ileum and colon are continuous although the histologic make-up is distinct. The colon and the rectum are separated by a well developed sphincter, as are the rectum and the cloaca. Lesions in the colon usually developed first in the region just above the sphincter.

lesions involved chiefly the superficial parts of the mucosa. Tissues included in them were changed to a dull-colored blood-stained friable mass, and their peripheries were marked by narrow hyperemic zones. Slight or moderate congestion and edema of the adjacent tissues accompanied the small ulcers which, with their bright red-brown borders, stood out prominently.

As a rule, death did not occur while the disease was limited to individual ulcers. Instead, the small lesions apparently enlarged rapidly and involved large areas of the mucosa and underlying tissues. Usually, when animals died of the disease, the entire mucosa of the colon and adjacent portions of the ileum were converted into dull-colored blood-

stained tissue and covered with masses of soft, friable blood-stained exudate, which sometimes formed a cast of the lumen. The wall of the intestine was thickened, intensely congested and inelastic. The serous surface was dull slate gray or grayish red, mottled by dull red-brown and red-purple areas, with blood vessels prominent (fig. 2*A*). Many variations of these changes were encountered. Occasionally death occurred when only the distal half of the colon had undergone ulceration. And, while ulceration of the mucosa of the terminal portion of the colon was found only in animals that were put to death after inoculation, several animals died while lesions were still composed of individual ulcers. In these animals ulcers were distributed over much of the epithelial surface, and hemorrhages apparently terminated the disease at this stage, the lumen of the intestine being filled with freshly clotted blood.

Usually pathologic changes did not occur distal to the colon, but occasionally, when extensive and severe lesions had developed, the rectum was involved also, then showing both discrete ulcers and diffuse necrosis. The mucosa of the cloaca was not involved in any animal.

In some of the animals in which spontaneous infection was found, edema and congestion of the wall were more marked than in any of the animals with experimentally induced disease. The boas (Constrictor constrictor and Epicrates angulifer, table 1) and the king snakes (Lampropeltis getulus, table 1) presented the following appearances: The muscles of the body wall about the large bowel were stained with blood. The serous coat of this part of the intestine was composed of a layer of turbid jellylike substance, streaked with blood. The wall of the intestine was thickened to four or five times the normal. The cut surface was dull dark red-brown, the lumen was narrowed to a slit, and the mucosa was dull dark brown and almost dry.

Small Intestine.—As has been indicated, pathologic changes in the mucosa of the distal part of the ileum were often continuous with and similar to those in the adjoining colon. Usually a portion of the terminal part of the ileum from 5 to 10 cm. in length was involved. The ulcers in the distal part of the segment were confluent; those in the midportion tended to become separated, and those in the upper part were scattered and small (fig. 2*A*).

In those animals that have been recorded as having had amebiasis of the small intestine, the pathologic changes in this part of the intestinal tract were continuous with those in the colon, and did not differ from them except in severity. The serosa and wall had undergone changes similar to those in the colon. The mucosa was dull dark red-brown with a thin broken layer of pale brown exudate covering tips of villi or crests of folds in the membrane. Blood-stained mucus, in which were sus-

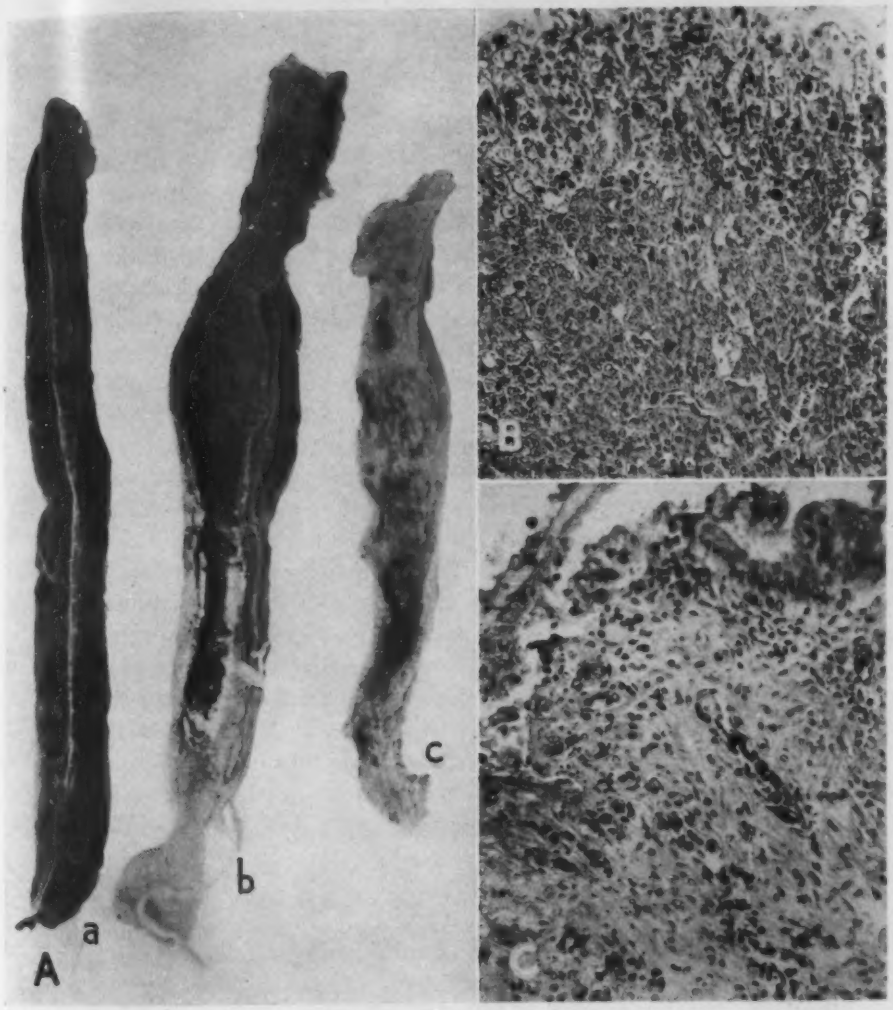

Fig. 2.—*A*, liver (*a*), colon (*b*) and stomach (*c*) of a garter snake (Thamnophis sirtalis), the body of which was about 80 cm. in length. These organs are shown in the natural size. Parts of the liver are covered by mesentery. The irregular breaks on the surface of the liver were caused by handling. The longitudinal groove is the portal vein. The mottled appearance which one sees here is typical of amebic infection; the more lightly colored areas are necrotic foci. Attached to the colon are the ileum, rectum and one kidney with its ureter, to indicate anatomic relations. Note that the wall of the colon does not spread when cut. It is thickened, and exudate partially covers the mucosa, especially in the lower segment, but is absent in the rectum. The sphincter between these parts is just distal to the end of the mass of exudate in the colon. The disease is less severe in the upper part of the colon and ileum, much less exudate lies in the lumen, and the wall is not so greatly thickened, although the lumen is widened. In the stomach scattered ulcers are seen in the upper half of the mucosa, while in the lower half lesions are more numerous but do not involve the pylorus. *B*, superficial ulceration of the gastric mucosa in Natrix rhombifera (experimental animal); hemalum and eosin; × 150. Four amebas lie among degenerated tubules. The inflammatory response about a lesion of this type was usually inconspicuous. *C*, border of a relatively large ulcer in the colon of Natrix cyclopion (33, table 2); hemalum and eosin; × 200. The epithelium and underlying tissues are undergoing necrosis, but the inflammatory response is relatively slight. Three amebas lie in the tissue beneath the epithelium, in the upper right quarter of the field.

pended small masses of exudate, filled the lumen. Cysts and trophozoites usually occurred in large numbers in this fluid, but their numbers decreased with postmortem changes.

Stomach.—Early stages in the formation of ulcers in the stomach were represented by circumscribed areas about 2 mm. in diameter and 1 mm. in depth, in which the tissue was changed to dull, pale brown friable material. These foci were slightly depressed below the level of other parts of the surface, were outlined by narrow zones of hyperemia and occurred at random. Other parts of the organ were unchanged. Increase in size and number of ulcers was accompanied by more pronounced inflammatory response. All layers of the wall became thickened through edema and congestion. The mucosa was dark dull red-brown and covered by blood-stained mucus, in which masses of exudate were suspended (fig. 2*A*).

Individual ulcers were usually less than 2 cm. in diameter. They rarely fused, although numerous and widely distributed. Usually their outlines on the mucosa were circular, and they extended into the mucosa and submucosa as cone-shaped areas of necrosis. Soft, friable blood-stained masses of exudate and degenerated tissue commonly filled the cavities and sometimes extended above the epithelial surface. In a few cases, there were no distinct ulcers, but wide areas of the mucosa were dark dull red-brown, thickened and necrotic, with masses of blood-stained exudate on the surface. Amebas could always be recovered from this exudate and from the underlying tissues.

p_H of Gastric Contents of Natrix.—That disease of the stomach increased as the interval between inoculation and death was prolonged was noted. During the development of the infection food usually was not taken, the stomach possibly did not form acid, and intestinal fluids may have been more or less static. The following observations appear to have some bearing on the problem: Mucus and tissue scraped from the gastric mucosa of ten specimens of Natrix which were killed ten days after their last meal were suspended in 0.5 per cent saline solution. By the colorimetric method the average reading for this material was p_H 7.4. Almost identical were records on material from the stomachs of ten specimens of Natrix which died of amebiasis but in which the infection had not spread to the stomach, and examination of an equal number of ulcerated stomachs gave similar results. Suspensions of equal parts of material from the stomachs of these animals and culture medium supported growth of E. invadens as well as did unchanged medium. Partially digested food, taken from the stomachs of five specimens of Natrix which were killed about twenty-four hours after feeding, and suspended in 0.5 per cent saline solution, was distinctly acid, varying from p_H 3.8 to 4.2 and averaging p_H 4. Trophozoites of E. invadens had stopped movement and were undergoing fragmentation within fifteen minutes after exposure to equal parts of culture medium and gastric contents from this group of snakes.

Liver.—Whether or not initial stages of the disease may affect one part of the liver more frequently than another has not been determined.

Except for one snake, in which lesions were confined to the cephalic pole, there has been either diffuse disease or none at all.

The diseased liver was always mottled, dull pale brown and dull dark red-brown, or intermediate shades of these colors. The organ commonly seemed swollen and was easily torn. On the capsular and cut surfaces, dull pale brown tissue occupied about a third or a fourth of the organ, being distributed in irregular circular or oval areas, irregular bands or broken networks and interspersed by continuous networks of darker parenchyma. Usually there was some similarity to the mammalian liver that shows passive congestion. In occasional cases, complete segments of the organ were colored dull pale brown except for a narrow zone of tissue about the portal vein (fig. 2*A*). The capsule of the liver was thickened in only a few instances.

Areas of necrosis in the livers of monitor lizards varied in size from foci which were just visible to masses 3 or 4 cm. in diameter. In these lesions the tissue was changed to dull pale brown friable material, sharply outlined in the parenchyma. In contrast to the regular arrangement of necrotic areas in the livers of snakes dying of amebiasis, these lesions were irregularly scattered.

HISTOLOGY

Histologic changes which were associated with invasion of tissue by E. invadens were studied in material from as many animals as possible. In some cases postmortem degeneration of the bodies so obscured detail of tissue arrangement that sections were worthless, but in many of the animals autopsies were made soon after death, and apparently disease processes were not affected by autolysis. However, unless otherwise indicated, the descriptions that follow apply to tissue specimens taken from specimens of Natrix which had been killed. Small lesions were studied in serial sections.

Gastro-Intestinal Tract.—The smallest and perhaps the earliest lesions encountered were superficial areas of necrosis where epithelium and supporting tissues had been changed into granular eosin-staining material (fig. 2*B* and *C*). A few specimens of E. invadens, rarely more than five or six, usually lay among the degenerated cells or in the tissues just beneath the necrotic focus. These organisms were accompanied by bacteria of various morphologic types. Epithelial cells bordering the ulcer were either shrunken and separated or moderately swollen and elevated from the basement membrane. Tissue elements immediately underlying the lesion were separated by edema, and capillaries were distended, but at this stage leukocytes usually had not accumulated in notable numbers.

Slightly larger ulcers seemed to have been formed by expansion of the initial ones in all diameters, and, as would be expected, the inflam-

matory reaction increased in intensity as the lesions enlarged. About equal numbers of granular and nongranular leukocytes accumulated along the inner margin of the necrotic area and entered the degenerated tissue. Capillaries adjacent to the focus sometimes were blocked by hyaline thrombi or were eroded, and erythrocytes were mixed with material in the ulcer and with the eosinophilic coagulum which usually covered the eroded surface in a lesion of any size. Lymph channels were widened, and supporting tissues of the mucosa were separated by edema. Amebas and accompanying bacteria were relatively more numerous in the larger ulcers, being distributed through the débris of the lesion and in the border of leukocytes or in lymph spaces adjacent to this border.

In the stomach, ulcers usually remained as isolated lesions involving sharply limited areas in the mucosa and submucosa. These lesions were outlined by relatively wide zones of leukocytes among which nongranular cells predominated, and the bordering lymphatics and blood vessels were distended. Extension of the necrotic foci into the muscular coats was not encountered.

Lesions in the small intestine commonly involved the superficial parts of the mucosa until rather widespread, and even in the cases in which involvement of this part of the intestine was severe, necrosis extended no deeper than the submucosa (fig. 3 *C*).

With further development of the disease in the colon, the outlines of individual ulcers were lost in widespread necrosis of the mucosa. In addition to completely destroying the inner layer of the intestinal wall over relatively wide areas, the process extended irregularly into the submucosa and muscular coats, so that in many places only the serosa remained intact and it was thickened by edema and infiltration of granular and nongranular leukocytes (fig. 3 *A, B* and *D*). Sometimes all of the degenerated tissues were converted into granular eosin-staining material, while in other lesions only the outer parts of the mucosa underwent this change, and in the deeper tissues, although apparently necrotic, outlines of blood vessels and other structures were recognizable.

In addition to the blockade of capillaries by hyaline thrombi, occlusion of larger blood vessels occasionally took place as individual ulcers destroyed the mucosa. And with extension of the inflammatory changes, the walls of many arteries and veins were invaded by leukocytes and their lumens blocked by thrombi. Occasionally, wedge-shaped necrotic areas which extended deep into the intestinal wall were formed about thrombosed vessels.

Amebas became more numerous in the extensive lesions; some were scattered through the necrotic tissue, but most of them accumulated near the inner boundaries of degenerated areas and lay among the

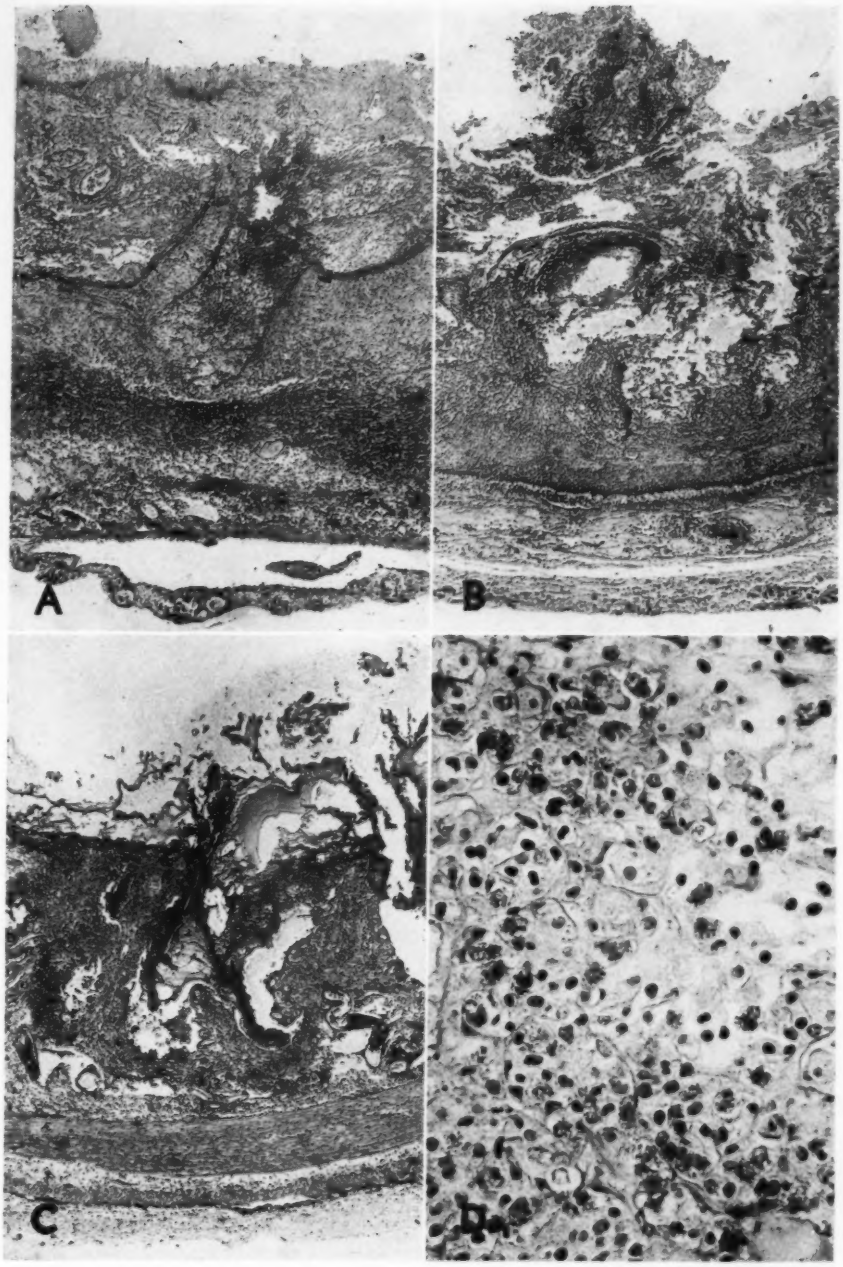

Fig. 3.—*A*, colon of Natrix rhombifera (12, table 2), showing advanced disease; hemalum and eosin; × 30. Necrosis involves all layers of the wall, except the serosa, which is densely infiltrated by leukocytes. *B*, colon of Natrix rhombifera (case 9, table 1), showing advanced disease following spontaneous infection; hemalum and eosin; × 30. Necrosis of the wall is sharply limited by a zone of nongranular leukocytes. *C*, small intestine of Lampropeltis getulus (case 5, table 1), showing advanced disease following spontaneous infection; hemalum and eosin; × 30. Necrosis is limited to the mucosa and submucosa. *D*, a portion of the serosa shown in figure 3 *A*; hemalum and eosin; × 300. Granular and nongranular leukocytes, among which lie numbers of amebas, obscured the normal tissue. The zone shown in this figure marks the inner limits of the lesion.

massed leukocytes or in lymph and blood channels (fig. 3 *D*). When tissues were fixed some time after death, however, the amebas were found in the deeper, intact tissues, leaving the necrotic areas almost free (fig. 4 *A*). E. invadens often was less numerous in the water snakes (Natrix) than in other animals.

In a few instances sections of the wall of the large intestine of the water snake (Natrix) and other animals contained relatively deep, sharply outlined ulcers, the inner walls of which were formed by irregular narrow layers of acidophilic material, and immediately beneath this and mingled with it, a dense mass of leukocytes, chiefly nongranular cells, outlined the break in the tissue. The leukocytic border merged rather abruptly with surrounding structures, and in this region capillaries were numerous and distended. Cavities formed by these ulcers sometimes were almost free from degenerated cells and from exudate, and amebas were rarely found in them.

In other instances necrotic parts of the intestinal wall were separated from underlying structures by narrow, sharply outlined borders of nongranular leukocytes. These cells usually assumed a palisade formation (figs. 3 *B* and 4 *D*).

Liver.—At an undetermined stage in the development of intestinal disease E. invadens entered the blood stream and was carried to the liver. When snakes were killed while hepatic lesions were in the early phases of formation, small numbers of amebas were found in the lumens of lesser branches of the portal vein and of the capillaries. In the veins the parasites sometimes were associated with emboli that were made up of blood clot and leukocytes.

The earliest recognized changes in the hepatic parenchyma developed about small groups of amebas that had collected in capillaries or had entered the columns of hepatic cells. In an irregularly outlined focus about the parasite the liver cells were swollen and stained dully basophilic in contrast to the lighter color of the surrounding tissue. Capillaries in the area were partially compressed but contained considerable numbers of both nongranular and granular leukocytes (figs. 4 *B* and *C* and 5 *A*).

Larger, apparently more advanced lesions that formed during the earlier stages of hepatic disease seemed to be expansions of smaller ones, without evident relation to tissue architecture. Their outlines were irregular and were often marked by hemorrhage into a zone of swollen, partially degenerated cells, among which occasional amebas were usually found. Leukocytes accumulated chiefly at the border of these foci. In the more central parts of these areas the liver cords and capillary walls were reduced to cell fragments or masses of eosinophilic coagulum.

With advance of intestinal disease, larger and more numerous emboli apparently were brought into the liver from the vessels of the intestinal

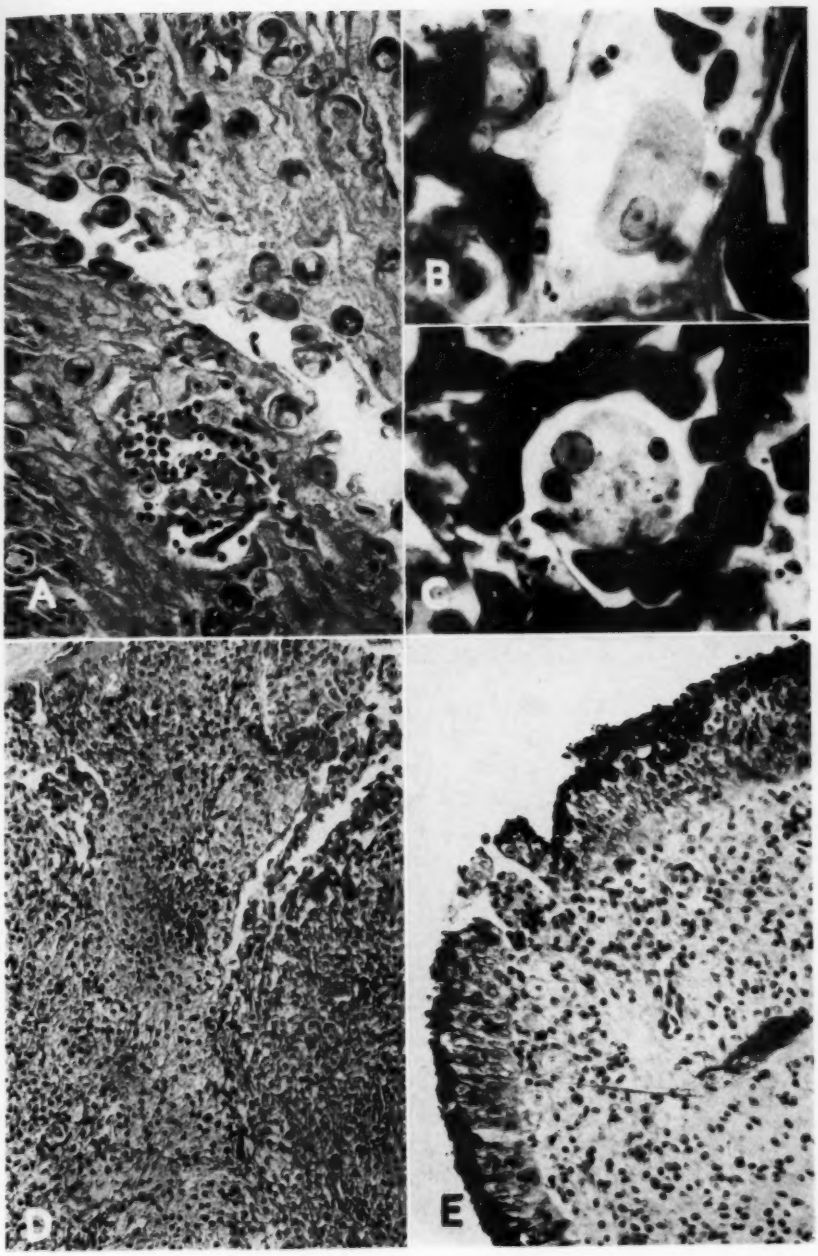

Fig. 4.—*A*, an area in the intestinal wall in figure 3 *C*, showing postmortem invasion of lymph channels by amebas; some of these appear to have encysted; hemalum and eosin; × 300. *B* and *C*, liver of Natrix rhombifera (9, table 2) infected by E. invadens; iron hematoxylin; × 1,000. *B* shows an ameba in a capillary. Note the pseudopod. In *C* the ameba is in a focus of necrosis, and its cytoplasm contains tissue nuclei. *D*, healing (?) ulcer in the colon of Natrix rhombifera (2, table 2); hemalum and eosin; × 120. The lesion is outlined and filled by nongranular leukocytes. *E*, healing (?) ulcer in the colon of Natrix sipedon (39, table 2); hemalum and eosin; × 200. The break in the epithelium is filled by nongranular leukocytes. Two amebas, both closely surrounded by phagocytic cells, lie to the left of the ulcer, beneath the epithelium. Nongranular leukocytes are numerous in the tissue.

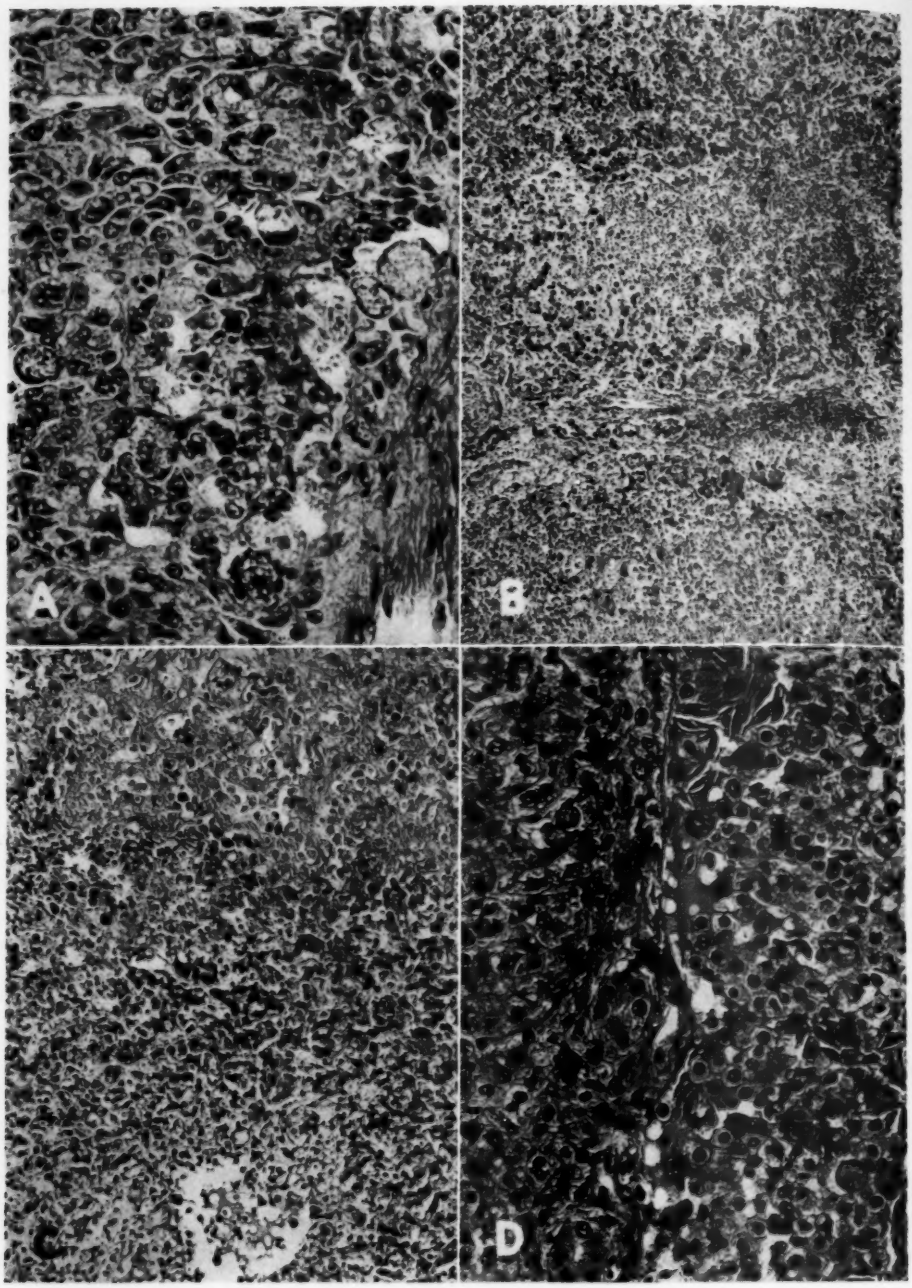

Fig. 5.—*A*, liver of Natrix rhombifera (12, table 2); hemalum and eosin; × 300. Note amebas in the hepatic cords and capillaries adjacent to the wall of a branch of the portal vein. Many cells are undergoing degeneration, and occasional leukocytes have collected in the area. *B*, liver of Natrix rhombifera (1, table 2); hemalum and eosin; × 100. Note thrombosis of a branch of the portal vein and foci of necrosis in the adjacent tissue. These changes were found regularly in livers of snakes that died of amebiasis, but relatively few amebas were associated with them. *C*, healing (?) lesion in the liver of Natrix rhombifera (10, table 2); hemalum and eosin; × 140. Note the portal vein at the lower left surrounded by phagocytic cells, some of which extend into the lumen. A necrotic focus is sharply outlined, and the nuclei in the liver cells about it stain darkly. (Compare with *B*.) *D*, liver of Natrix rhombifera (10, table 2); hemalum and eosin; × 300. Nongranular leukocytes with occasional polymorphonuclear cells invade a thrombus in a branch of the portal vein. Note that amebas lie among the leukocytes and are closely surrounded by them.

wall, and these were accompanied by relatively greater numbers of amebas. Larger branches of the portal vein were occluded by the blood clots, or amebas invaded the walls of blood vessels, stimulating infiltration of this tissue by large numbers of leukocytes. Occasionally nongranular leukocytes replaced the intima of branches of the portal vein and partially blocked the lumen.

With these changes in the blood supply of the liver, necrosis of the tissue was often widespread, the degenerated areas forming irregular networks, with islands of liver tissue remaining only about the portal zones of occasional lobules (fig. 5 *B*). And, in sections through the longitudinal axes of occluded vessels, necrotic foci seemed more numerous in adjacent lobules than elsewhere in the tissue, suggesting that interference with the blood supply aided their formation.

Some of the necrotic foci in the livers of specimens of Varanus salvator and Natrix rhombifera (5, table 2) differed from the usual type in that boundaries were formed by layers of nongranular leukocytes. These borders were from 2 to 10 or more cells in thickness, and peripheral to this wall parenchymal cells were intact. This reaction is similar to that which occurs about other lesions, such as tubercles or encysted parasites, in the tissues of reptiles.

Atypical Lesions.—In some of the experimental animals which were killed we encountered certain atypical lesions (table 2). Some of these were recognized on gross examination; others were found when sections had been prepared. Altogether they suggested reparative processes.

The mucosa of the colon of one snake (2, table 2) was eroded by scattered, apparently progressive small ulcers, which in sections seemed typical of the disease. In addition, lesions of different histologic make-up were also found. In the latter a compact mass of nongranular leukocytes replaced a part of the supporting tissue of the mucosa and filled a break in the epithelial surface, above which these cells joined a small mass of exudate (fig. 4 *D*). Epithelium about the edges of these ulcers was arranged irregularly, and the cells were flattened as might be expected at the border of a healing wound.

Amebas were not associated with these lesions, but in another snake (39, table 2) occasional ones lay among the nongranular leukocytes which formed the floor of smaller ulcers (fig. 4 *E*). In this snake as well as in three others (28, 37 and 38, table 2) gross evidences of disease in the colon consisted of small areas in the mucosa in which capillaries were engorged. Small masses of blood-stained mucus covered these foci, and on microscopic examination of this material in wet smears occasional amebas were found. Sections through these injected areas presented varied pathologic changes. In one animal (39, table 2) several small ulcers, tiny breaks in the mucous surface, were found (fig. 4 *E*). The epithelium bordering these was normally arranged except for a few cells at the immediate edge of the lesion. Nongranular leukocytes had accumulated in the underlying tissues, and a compact mass of these cells filled the break in the epithelium. Association of amebas with these lesions has been mentioned. In two others of these animals (37 and 38) sections did not reveal ulceration. However, considerable numbers of nongranular leukocytes had infiltrated the supporting

tissues of the colon, and scattered amebas lay among these cells, often surrounded by small groups of them. Amebas were not located in sections of the wall of the colon of snake 28, nor had leukocytes accumulated in any numbers. But, in the epithelial surface, small groups of cells were shrunken and their nuclei pyknotic. Strands of mucus in which erythrocytes were suspended seemed to pass from these foci into the lumen.

The entire alimentary tract of snake 10 (table 2) was normal on gross inspection, but in sections of the stomach a loosely arranged mass of granular and nongranular leukocytes replaced a microscopic area in the submucosa. Amebas were relatively numerous in the central part of the focus. The overlying mucosa was intact in serial sections.

The livers of snakes (10, 28, 37, 38 and 39 as well as that of snake 11, table 2) contained fewer necrotic areas than was typical of the disease. In sections, lesions varied in extent from areas involving several lobules to small intralobular foci. Usually, the outlines of these areas seemed sharper than those in other animals and the degenerated mass more compact and more closely surrounded by well stained liver cells, with cords of darker staining, possibly regenerating cells at the periphery (fig. 5 *C*). The intima of many branches of the portal vein was thickened by accumulation of nongranular leukocytes, or the lumen was partially filled by these cells, which infiltrated masses of coagulum (fig. 5 *D*). Amebas lying within the blood vessels of the liver did not stain as distinctly as those in tissue of other animals.

Amebiasis Compared to Bacterial Disease of the Intestine and Liver.—In our experience the changes due to infection by E. invadens were rarely simulated by bacterial diseases of the digestive tract and liver. During this study we examined more than a hundred reptiles that died of acute gastro-enteritis and hepatitis of bacterial origin. Of these, fifty-four were representatives of common groups of snakes (eighteen, Natrix sp.; eleven, Lampropeltis sp.; six, Agkistrodon sp., and nineteen, Crotalis sp.) and are believed to have supplied adequate material for comparing types and distribution of lesions due to amebas and bacteria.

Among these fifty-four animals, pathologic changes developed in the stomach in twelve, in the small intestines in twenty-eight, in the large intestine in fourteen and in the liver in ten. In only four of the snakes were the liver and all segments of the intestines diseased simultaneously.

In gross appearances the lesions in the stomach and small intestines usually were similar to those associated with amebic infection, except that the deep ulcers of the gastric mucosa and submucosa that developed in some of the experimental animals were not reproduced. In the small intestines, in contrast to amebiasis, bacterial disease produced the most severe changes in the upper and middle segments rather than in the terminal part of the ileum.

Inflammation of the wall of the colon was less pronounced than that of the small intestine. This part of the intestine was usually not thickened, although the mucosa was diffusely injected. When ulcers developed, they were limited to the superficial tissues and were not numerous.

Lesions in the livers of these animals consisted of distinctly separated abscesses or foci of necrosis, distributed throughout the organ. In no case was the reticulated or mottled appearance of amebic disease found.

In those animals from which tissues were taken for microscopic study, the chief difference, and perhaps the only difference, between amebiasis and bacterial disease was in the severity of the pathologic changes, which were much milder in the absence of E. invadens.

COMMENT

In Natrix, experimental infection by E. invadens regularly leads to death after a variable period of incubation, which in our experience has ranged from thirteen to seventy-seven days. During the interval between inoculation and death, cysts and trophozoites of this ameba are formed in the colon and rectum and may be passed in fecal material in the early stages of the disease. These cysts are fully capable of maturation, and if deposited in drinking water they would be a potential source of infection. Thus, spontaneous spread of the disease seems easily possible, and if a naturally infected snake were introduced into a group that was confined to a relatively small space it might readily serve as the nucleus of an outbreak of amebiasis such as those which have been described in this paper.

However, we have examined as controls over two hundred freshly collected water snakes (Natrix) in the course of our experimental work and have failed to demonstrate any case of natural infection by E. invadens. Nor has the ameba been found in the intestinal canals of frogs which are sometimes fed to water snakes in captivity and which are a natural food of these reptiles. Hence we know practically nothing concerning the incidence of E. invadens in wild or in freshly captured specimens of Natrix.

Nevertheless, natural infection apparently occurs, for amebiasis has developed in two groups of water snakes that were being exhibited in entirely different types of enclosures, one indoors and the other outdoors, and the outbreaks occurred as isolated events. In each instance, freshly collected snakes were introduced into cages in which no infected animals had been found previously, yet after about three months amebic disease developed in some of the snakes, and the incidence within each group was roughly proportional to the crowding.

The occurrence of amebiasis among reptiles other than water snakes remains unexplained. Our evidence on the kind of disease, presented in foregoing sections, and on the morphologic character and life cycle of two strains of amebas isolated from Natrix rhombifera and Varanus varius (Geiman and Ratcliffe, 1936) and of other strains isolated since that time indicates that the causative organisms of the disease in all cases belong to one species. Consequently, if spontaneous infections are as common among water snakes in zoological gardens as they seem to be, workmen, in cleaning cages, might easily carry mature cysts on wet brushes and brooms from an infected cage to cages containing other species of susceptible reptiles.

In our experience, the reactions of tissues of reptiles to invasion by E. invadens have not been essentially different in character from those which have followed infection by certain bacteria. These bacteria have

not been identified, only their morphologic character being determined, and, invading as they do the tissues of reptiles that have been in captivity for varying periods, they may not produce inflammatory changes that are identical with those which would occur in newly captured, presumably normal animals.

In certain respects, amebiasis of reptiles is comparable to amebiasis of mammals. Intestinal lesions in both types of vertebrates originate on the mucosa and extend directly into the deeper layers of the intestinal wall. Degenerative changes seem to be due to lytic action of the parasites on the tissues. Undermining ulcers with irregular edges, such as occur in human amebiasis, have not developed in the colons of reptiles. Extensive and deep involvement of an entire region of the intestine is most frequently noted, and in this respect the disease is similar to the experimental infection in young cats. Perhaps these differences may be related to differences in anatomic make-up and relative size of organs in reptiles and mammals.

Involvement of the liver in amebiasis is much less common in mammals than in reptiles, but here again anatomic and physiologic differences may be the explanation. Since involvement of the colon in reptiles is extensive and deep, the amebas gain access to the blood stream, portal circulation and liver early in the infection and initiate disease first by invasion of the parenchyma, causing necrosis, and by invasion of the walls of blood vessels, with formation of thrombi. However, in the animal that has died of amebic infection, the blood supply of the liver is interfered with to such an extent by emboli that the changes caused by amebas are largely obscured.

Lesions of the upper part of the small intestine and stomach are peculiar to the reptilian disease and may be due to the inactivity of the stomach of animals which refused food under conditions of captivity with the result that gastric fluids become favorable for the growth of amebas and invasion of the wall of these segments of the intestine. This condition is not likely to occur in warm-blooded animals, because frequent feeding is more necessary for their survival. To our knowledge, no amebic lesions of the stomach and upper part of the small intestine have been described in mammals.

In contrast to the numbers of organisms found in lesions of mammalian amebiasis, relatively small numbers of amebas are associated with lesions in reptiles. Occasionally E. invadens may be demonstrated only by culture or by prolonged examination of teased or sectioned tissue in advanced stages of the disease. However, these cases are rare, but grossly they may be mistaken for bacterial infection.

From our material we cannot be certain that reptiles ever recover spontaneously from infection by E. invadens. There were, however,

certain lesions which seemed to be undergoing repair, but these were found in animals that had been killed well within the possible incubation period of the disease. Furthermore, occasional ulcers in the colons of animals that died of the disease showed much the same cellular response. In only one snake was there complete loss of the infection, and perhaps in the other cases of seeming partial recovery there was merely temporary readjustment of the host to the parasite.

SUMMARY

We have observed spontaneous amebiasis in thirty-two reptiles of the families Varanidae, Scincidae, Colubridae and Boidae and have obtained experimental amebiasis in three species of Natrix (Colubridae) by feeding mature cysts of amebas which were isolated from Natrix rhombifera and Varanus salvator. Anolis carolinensis and Alligator mississippiensis apparently were not susceptible to infection.

After inoculation with E. invadens all of the water snakes (Natrix) refused food for about two weeks, and the greater number of them died of amebiasis without again having taken food. In the early stages of this disease, from ten to fifteen days after receiving the amebas, occasional animals were seen also to pass bloody mucus by rectum. Other than these manifestations, amebiasis has not been accompanied by recognizable signs of illness in those reptiles in which we have studied the disease.

The interval between inoculation and death has varied from thirteen to seventy-seven days in Natrix, but the extent and severity of the pathologic changes were not related to the length of the incubation period.

In many cases amebiasis of reptiles was a disease of the colon and liver, but lesions also developed in the stomach and small intestine. Invasion of the mucosa of the stomach and small intestine usually seemed secondary to infection in the colon, but in several instances lesions were confined to the stomach and liver, and in one animal only the stomach was involved.

Pathologic changes in the colon, small intestine and stomach were initiated by invasion of small areas in the superficial parts of the mucosa by E. invadens. In these areas tissues underwent necrosis, and this was accompanied by acute inflammatory response. Lesions in the colon extended to include much of the mucous surface and invaded the submucosa and muscular coats of the intestinal wall. The walls of many blood vessels were involved by inflammatory changes, and thrombi formed in their lumens. Amebas entered both blood and lymph channels. Necrosis of the mucosa of the small intestine often was as widespread as in the colon, but degenerative changes usually did not extend deeper

than the outer parts of the submucosa. In the stomach lesions usually remained as individual ulcers.

The amebas entered the liver through the portal veins and were found in the capillary bed of the organ, in the walls of blood vessels and in the parenchyma. Many branches of the portal vein were blocked by thrombi, which were associated with invasion of the walls of the vessels by the amebas, or by emboli from thrombosed vessels in the intestinal wall. Degenerative changes in the liver parenchyma usually seemed to be more closely related to obstruction of blood vessels than to the presence of amebas but were initiated by the amebas.

We suggest that: (1) The lesions of the digestive tract are initiated by lytic action of the amebas (E. invadens) but that the characteristic changes are due to the combined effects of the action of amebas and bacteria and to thrombosis of blood vessels. (2) The amebas also cause focal necrosis of the hepatic parenchyma, but obstruction of branches of the portal vein by thrombi and emboli lead to massive necrosis of the liver and obscure the effects of the amebas.

MULTIPLE MESENCHYMAL HEMENDOTHELIOMA

REPORT OF A CASE

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NEW YORK

The occurrence of multiple vascular tumors has been interpreted in different ways. Some authors assert that all the neoplasms in the same patient owe their origin to a general endothelial disturbance. A second group is of the opinion that such coincidental multiple tumors are of congenital origin, while a third group, not denying the claims of the others, points out that metastasis may be responsible. The study of the case reported here, briefly described elsewhere,[1] tends to substantiate the view of the third group.

The endothelial cell is the basic unit, and its varied physical changes in the various neoplasms, to be described, help clarify the histogenesis of tumors with such different names as angioma, endothelioma and hemangio-endothelioma. It will be noted that these studies emphasize the intimate relationship of endothelium and mesenchyme.

REPORT OF CASE

A 62 year old truckman was admitted to the New York Post-Graduate Medical School and Hospital Aug. 2, 1934. He complained of loss of sensation below the diaphragm, inability to urinate and defecate and pain in the armpits, groins and thighs. In the preceding April he had a cold for several weeks, which left him weak, exhausted and suffering from aches and pains, particularly in the legs. A month later constant moderate pain developed in the right groin and within two weeks in the left. The pain continued until three days before admission when all sensation in the lower limbs disappeared. A limp was noted a few weeks before, which became progressively worse. Four days before entering the hospital the patient wabbled while walking and the same afternoon lost control of his legs, while control in the upper limbs was unaltered. Constipation and dysuria were also noted, and later catheterization was necessary. There was a loss in weight of about 25 pounds (11 Kg.).

Examination at admission revealed complete flaccid paralysis of the lower limbs. The biceps, radial, triceps, ulnar and patellar reflexes were present or increased. The suprapatellar, upper and lower lateral abdominal, and cremasteric reflexes were absent. The achilles tendon reflex was noted only on the left side, and a Babinski sign was elicited bilaterally. Diffuse and localized tactile pain and temperature sensations were absent below the level of the second thoracic

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1. Geschickter, C. F., and Keasbey, L. E.: Am. J. Cancer **23**:568, 1935.

vertebra. The temperature was 101 F. (39 C.), with elevation of the pulse and respiration rates. The blood pressure was 114 systolic and 70 mm. diastolic. There were 3,220,000 red blood cells per cubic millimeter and 7.98 Gm. of hemoglobin per hundred cubic centimeters, with polychromasia, achromia and microcytosis. Of 10,400 white blood cells, 85 per cent were polymorphonuclear neutrophils. The butyric acid test of the spinal fluid (Noguchi's reaction) was 1 plus; of 25 cells, 80 per cent were lymphocytes; the complement fixation test for syphilis was negative. It was the clinical impression that the condition was transverse myelitis, probably of influenzal origin.

At the end of the first week in the hospital limited movement appeared in both legs and a plantar reflex in the right leg. Five weeks after admission two large nontender masses, progressively increasing in size, developed over the top of the head; a third was said to have been present at the onset of the illness. Sacral, scrotal and ankle edema appeared, concomitant with decrease in urinary output. Anemia became more marked, and the patient died September 17, with signs of pulmonary edema.

Necropsy.—At necropsy, begun two hours after death, there was generalized anasarca with bilateral hydrothorax. Bronchopneumonia, active as well as organized, was present in the emphysematous lungs. There was moderate atherosclerosis of the coronary arteries and aorta, but arteriosclerosis was not pronounced in the kidneys. The periprostatic venous plexus was thrombosed. The chief features were tumors in the iliac fossae and smaller tumors throughout the body.

In the right iliac fossa there was a large mass, 18 by 13 by 10 cm., with a broad base firmly fixed to the abdominal surface of the ilium. Lifted by the tumor were thinned pelvic connective tissues, muscles and fascia, forming a capsule, on which rested the nerves of the lumbar plexus. The mass was freed by cutting through the greatly thinned and disrupted iliac cortex at its periphery. It weighed 1,170 Gm. Through a 10 cm. defect in the ilium the examining finger entered the soft tissues about the right hip. The inner iliac cortex was more extensively destroyed than the outer surface. A neoplasm in the left iliac fossa, generally similar but smaller (15 by 12 by 6 cm.), had eroded only the abdominal cortex of the ilium.

The base of each iliac mass had a wide yellow-gray necrotic center and an irregularly narrow brick-red outer zone, where there were round to oval, generally radial spaces of varying size, distended with clotted blood. Some were separated from each other by slender strands of the encapsulating connective tissue, which dipped inward. The periphery, with zigzag inner border, was everywhere delimited from the large necrotic central area by sharp differences of color. The latter was grayish brown to yellowish brown, and the architecture was generally similar to that of the periphery. Careful search revealed minute fragments of bone in the necrotic center, as well as in and just beneath the capsule.

Numerous smaller neoplasms throughout the body were similar to the tumors of the iliac fossae. A neoplasm in the left temporal muscle had penetrated the underlying bone and invaded the dura mater, which was not adherent to the leptomeninges. The latter were without alteration and the brain was not involved. Multiple vertebral neoplasms narrowed the spinal canal at several points. Definite compression of the spinal cord, however, was seen only in the upper thoracic region and at the level of the fourth and fifth cervical vertebrae. There was invasion of the dura and of the spinal nerves, with compression of the cord.

Beneath the left visceral pleura there were several firm round reddish brown nodules. On the right side the lung was superficially involved in a neoplastic

mass, 6 by 4 by 3 cm., which in part replaced the fourth and fifth ribs and their muscles. The two leaves of the pericardium were separated with moderate difficulty because of recent adhesions. A massive growth, 9 by 6 by 5 cm., involving the right pulmonary hilar area, had invaded the wall of the right auricle and bulged into the cardiac chamber with resultant narrowing of the lumen of the auricle and of the adjacent superior vena cava. At several points smaller, subsidiary nodules lifted the auricular endocardium.

On the anterior surface of the right lobe of the enlarged liver (2,660 Gm.) there were two elevated neoplastic masses, the largest 5.5 cm. in diameter, covered by thinned hepatic capsule. The spleen, weighing 680 Gm., measured 17 by 12 by 5.5 cm. Two elevated nodules were present, one on the diaphragmatic surface and another on the gastric surface at the lower pole. Large tumors were also present in the sternum and in the perirenal fat, invading both adrenals.

Microscopic Examination.—The abdominal surface of the tumor in the right iliac fossa was covered by a capsule about 5 mm. thick, of moderately loose cellular, hyalinized, vascular connective tissue. In the capsule was a 3 mm. layer of nonlamellated bone with communicating marrow spaces of varying size. These were filled with loose, highly vascular fibrous marrow, which was continuous with the capsular connective tissue. There was active bone deposition, with less active bone destruction. The outer surface of this bone layer showed much active destruction. Even the osteoid tissue was in some places destroyed before it was changed to bone. On the inner surface, adjacent to the underlying neoplasm, there were osteoblasts and osteoid tissue.

The neoplasm proper irregularly invaded the connective tissue capsule so that broad interlacing septums were produced, incompletely subdividing the outer portions of the new growth into indefinite lobules. In these septums lay communicating trabeculae of bone, portions of which projected directly into the adjacent neoplastic tissue. The bone was generally nonlamellated, although lamellation was suggested in small fragments. The major portion of the trabecular surface was covered by osteoblasts, usually with a thin rim of osteoid tissue (fig. 1). The osteoblasts, particularly where the neoplasm impinged directly on bone, resembled the tumor cells to be described. The neoplastic cells directly adjacent to the osteoid tissue and similar to the osteoblasts had indefinite cell boundaries and were arranged in interlacing strands and whorls. Their oval, oat-shaped and spindle-shaped vesicular nuclei, however, were sharply outlined and deep staining. The spaces between the strands were crowded with red blood cells, few macroblasts, large mononuclear cells and immature polymorphonuclear leukocytes.

The central portion of this neoplasm, as well as of all the larger neoplasms throughout the body, was a faintly eosinophilic, delicately fibrillary reticulum, in which cell boundaries or membranes were rarely seen and then indefinitely. The interstices were of irregular size and shape and filled with blood cells of various types, separating the tumor cells. Some were small, from 1 to 2 red cells in width, round to oval, with distinct walls formed of an unbroken single layer of endothelial cells. In larger spaces, with maximum diameters of from 0.1 to 0.5 mm., the lining was one to two parallel layers of closely packed flattened cells with a smooth internal outline and an indefinite base. Other portions of their walls were more irregular in outline. Some spaces were several centimeters wide. Such cavernous areas communicated at points with the slender channels, suggesting that they arose as a result of dilatation and tearing of the latter.

The nuclei of the reticulum were generally of two types, much alike but differing in size, staining and density. One type, representing the practically unchanged endothelial cell (fig. 2 *a*), was a sausage-shaped or spindle-shaped cell, the contour

altering with the position of the cell. The nucleus was about 7 microns or less wide and from two to four times longer, moderately deep staining and faintly vesiculated. Some cells had each a single small nucleolus. The second type of nucleus strongly suggested that of the epithelioid cell when it was small and the mesenchymal macrophage (fig. 2 *b*) when it was larger. It was as long as the first type but several times as wide. Vesiculation was marked, as was the nuclear membrane, near which the fine chromatin network was thickened and prominent. From one to two nucleoli were encountered in many cells. There were relatively few mitotic figures. Isolated cells similar to fibroblasts were seen where the reticulum was more compact.

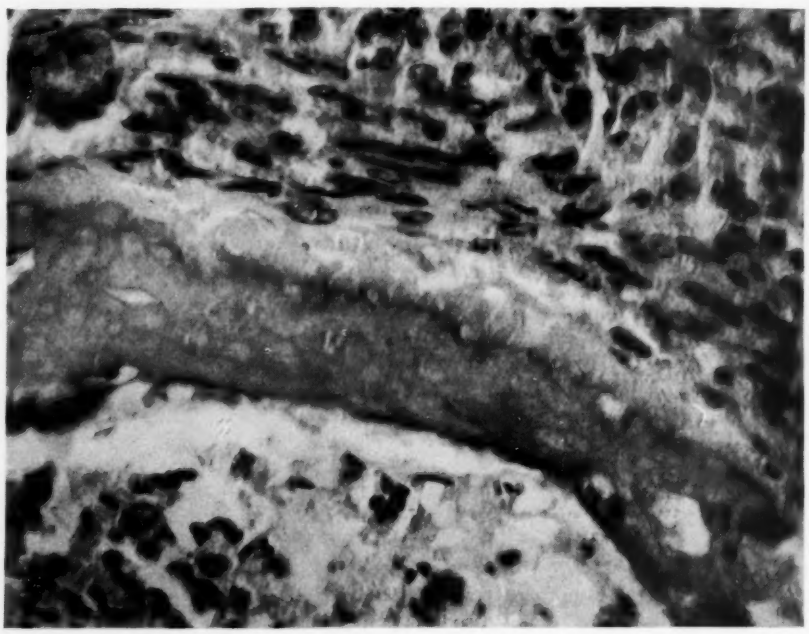

Fig. 1.—Malignant mesenchymal hemendothelioma of the right iliac fossa; × 450. A bone trabecula is shown, on which osteoid tissue is being deposited by osteoblasts which are generally similar to the adjacent neoplastic cells.

Isolated leukocytes, almost exclusively of the myeloid series, were virtually the only white blood cells in the reticular spaces. Among the many distinct types of white cells were polymorphonuclear leukocytes, neutrophils and eosinophils of varying maturity (fig. 2 *d*) with numerous staff forms. Others resembled myelocytes and premyelocytes (fig. 2 *c*), with relatively large deep-staining, irregularly round nuclei. The cytoplasm, a narrow rim about the nucleus, had fine granules, chiefly eosinophilic and basophilic. There were also many large oval mononuclear cells with oval nuclei. These arose from the lining of the spaces and finally floated free in the lumens. The first step in the process was seen when the nucleus of the lining cell became less vesicular and stained more deeply. As the cytoplasm assumed an eosinophilic hue, the cell was loosely attached to the fixed tissues. When it was free in the lumen it differed from the immature myeloid cell in its

larger size, in the oval shape of its nucleus and in the absence of cytoplasmic granules. That the precursors of the mature leukocytes were formed in the reticulum was confirmed by the absence of such cells in the larger spaces, where immature leukocytes were also scarce.

Erythroblasts were relatively rare compared with the myeloid cells. Macroblasts (fig. 2 *e*) and normoblasts were most common, but megaloblasts were not encountered in the sections examined. Hematopoiesis was seen most strikingly in the tumors of the iliac fossae.

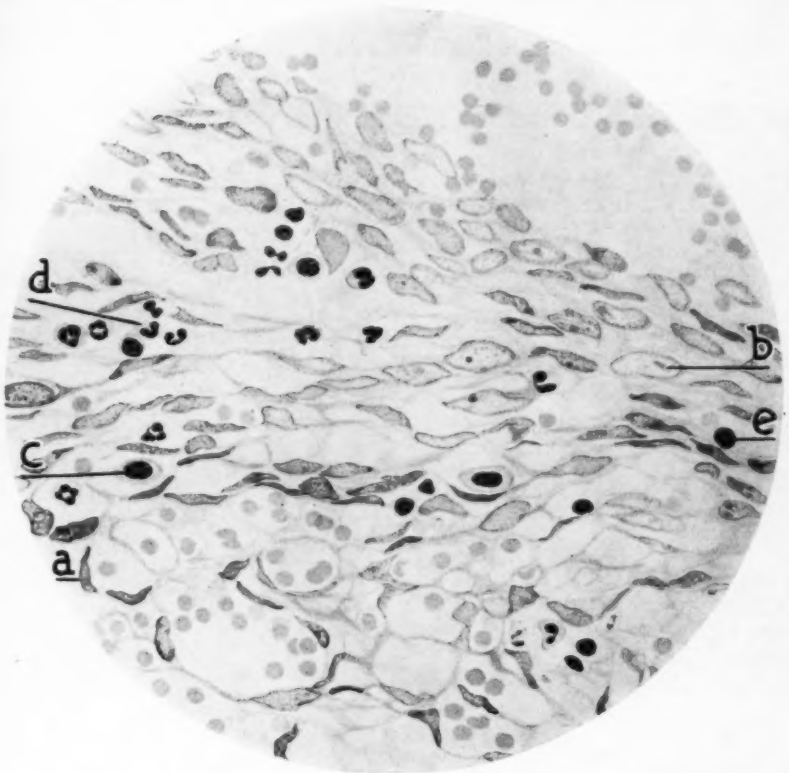

Fig. 2.—Drawing of malignant mesenchymal hemendothelioma of the right iliac fossa; × 1,000. The reticulum with its small and large interspaces shows two types of cells: the endothelial cell (*a*) and the mesenchymal cell (*b*). In the spaces are premyelocytes and myelocytes (*c*), immature polymorphonuclear leukocytes (*d*), macroblasts (*e*) and numerous red blood cells.

Collagen fibers, stained by the Van Gieson method, were not prominent. They were most readily identified in the tumor of the right iliac fossa and in the vertebral and hepatic neoplasms. In the latter the fibers merged with the reticulum of the adjacent vascular areas. The nuclei of the connective tissue were like those of the cells lining the blood spaces, the only difference being the flatter outline of the nuclei among the collagen fibers. Elastica formation was not recognized.

The metastases on the outer surface of the dura differed somewhat from the tumors of the iliac fossae. Cavernous spaces were fewer, and the closely packed neoplastic cells had spindle-shaped nuclei. These could not everywhere be distinguished from frank connective tissue cells where the neoplasm was irregularly subdivided by fibrous septums. The most striking features of the dural tumors were the whorl formations and the cells (fig. 3) forming them. The latter were round to polyhedral, densely arranged, with large prominent deep-staining nuclei and distinct nucleoli; many mitotic figures were seen. The cells resembled epithelial cells, suggesting the name "epithelioid," although it was clear they were similar to the endothelial tumor cells elsewhere. Round cells with prominent round nuclei lined some lumens in the dural tumor and might be called angioblasts.

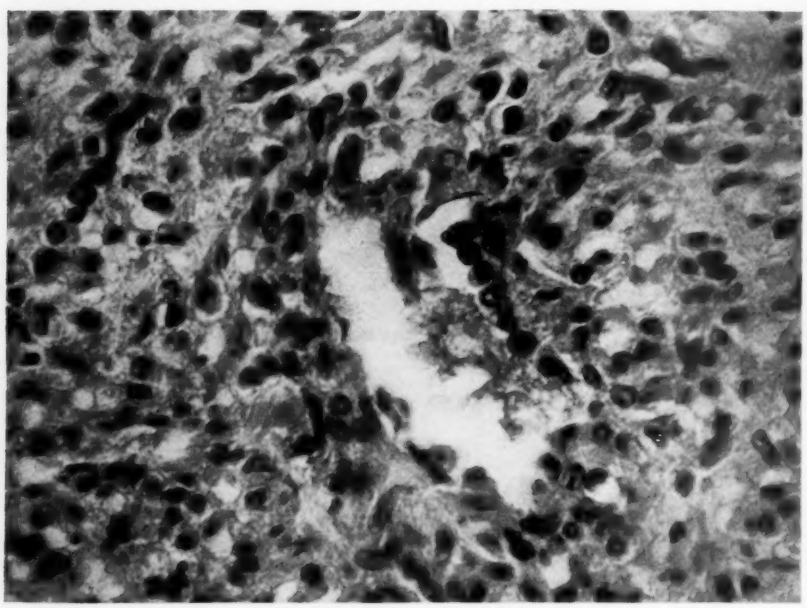

Fig. 3.—Dural metastasis of malignant mesenchymal hemendothelioma; × 450. The cells of the neoplasm might be described as epithelioid, and the prominent cells lining the right wall of the large central blood spaces as angioblasts.

The tumors in the liver and the endothelial response in that organ were especially interesting. Microscopically, as grossly, the neoplasm and the normal tissues were almost everywhere sharply demarcated, with isolated hepatic cells in the new growth near the junction. Adjacent to the tumor the hepatic cells were more or less atrophic. The nuclei of the sinusoidal endothelial cells were swollen and bulged prominently into the dilated capillary lumens. They were, however, not as large or as spindle-like as the cells of the neoplasm. A similar swelling was noted in the lining of the blood vessels of the portal areas. Many endothelial cells contained brown pigment granules. The sinusoidal lumens contained many red cells, a few erythroblasts, myeloblasts and immature polymorphonuclear neutrophils. In some places, however, there was no sharp transition from neoplasm to normal tissue. There was, instead, a wide zone in which slender disrupted hepatic cords

were still present, and the tumor cells insinuated themselves between sinusoidal endothelium and hepatic epithelium. Here the hepatic endothelial network resembled that of the neoplasm with but few differences; the hepatic endothelial cells showed a round and not a spindle shape, and the tissue network was not as dense as in the areas of frank tumor. Swelling of endothelial cells in the liver was seen as far as 6 mm. from the new growth.

The meninges and vertebrae, but not the spinal cord, were directly attacked by the neoplasm. The cells of the meningeal tumors were densely packed, with narrow intervening blood spaces, and had prominent deep-staining spindle-shaped to sausage-shaped nuclei. They were arranged in many places in interlacing bundles, which suggested the pattern of neurinoma. Collagen fibers were present in short, indefinitely delimited strands of varying thickness throughout the solid portions of the section and about larger blood spaces. With the Bielschowsky-Maresch silver stain [1a] there was everywhere in the neoplasm a reticulum similar to the meningeal connective tissue.

In the lung the metastases had irregularly shaped vascular spaces, some lined by spindle-shaped cells and others by large, swollen cells with deeply eosinophilic cytoplasm and large deep-staining vesicular nuclei. Each nucleus had a definite nuclear membrane and one or two distinct nucleoli. Some of these cells had desquamated into the lumens and contained brown to black pigment granules. Cell forms intermediate between the spindle cells and the large, swollen "epithelioid" cells and mesenchymal macrophages were also recognized.

In the right cardiac auricle the subendothelial cellular connective tissue was dense and hyalinized, with its deeper lamellae infiltrated by a thin zone of neoplasm parallel to the auricular surface. Beneath this the plane of the tumor pattern was tangential to, and more deeply was perpendicular to, the endocardial surface. The cardiac neoplasm had areas with cells more hyperchromatic and more densely packed together than elsewhere in the body. The nuclei closely resembled those of the dural metastases, with nucleoli and a prominent nuclear outline, with few mitoses.

A neoplasm in the spleen was surrounded by a wide zone of markedly congested pulp with numerous closely packed erythrocytes and many polymorphonuclear neutrophils, mostly immature. Only the cells of the few compressed cords partly surrounding the new growth had demonstrable endothelial hyperplasia. The splenic arteries and arterioles adjacent to the tumor, as well as those within the tumor but not yet destroyed, failed to show any swelling or proliferation of their endothelial linings. Within the tumor, which had few mitoses, the free cells, apart from red blood cells, were desquamated endothelial cells, some with hematogenous pigment grains.

COMMENT

The chief features of interest in the case reported are as follows: first, mesenchyme was recognized in both the undifferentiated and the fully differentiated connective tissue, bone, endothelium (in part lining blood spaces) and blood cells; second, the malignant character was demonstrated by active invasive spread within the organs of the primary site; third, there was also evidence that the widespread occurrence of neoplasms throughout the body could be attributed to true metastasis as well as to a multiple primary origin.

1a. Schmorl, G.: Die pathologisch-histologischen Untersuchungsmethoden, ed. 14, Leipzig, F. C. W. Vogel, 1928, p. 177.

One finds, as Maxinow,[2] among others, has pointed out, the different types of tissue arising from mesenchyme. It is well to remember that in the embryo cells are usually rounded only when in mitosis, while later they are of varied shape, mobile and arranged as a syncytium or network, with the interspaces filled with fluid or semifluid material. The anlages of the blood and vessel system are earliest seen in the blood islands, which later become tubular. Here the primitive blood cells seem to bud from the endothelium or are free in the vessel lumen. Mesenchymal cells, primitive blood cells and endothelial cells at this stage are very similar, later differentiating in special fashion. After the vessel system with its endothelial lining and circulating blood has formed, according to Maximow, true connective tissue appears in the mesenchyme, with the exception of the cells which later are to form cartilage, bone and smooth muscle.

The numerous tumors of this case represented mesenchyme and its derivatives, with the exception of cartilage and muscle. Mesenchyme was best seen in the prominent syncytium in the masses in the iliac fossae. As expected, the most primitive as well as the most differentiated characters were found at the primary site or sites, where multipotency was retained and more completely elaborated. This view is strengthened by the formation of bone by the tumor cells only in the right iliac fossa, for, as Erdheim [3] wrote, "if you can show this . . . can form bone, then the assumption that it arises from the bone cannot be doubted." The neoplasms in the iliac fossae further illustrate the role of mesenchyme in osteogenic tumors.

Collagen fibers were also found in the neoplasm, representing a late stage of mesenchymal differentiation. Further evidence of this late differentiation is furnished by Robb-Smith [4] in his study of explanted adult rabbit lungs. The sequence demonstrated by him was, first, production of mesenchymal macrophages from the alveolar interstitium, as seen in the present case, and, later, interstitial proliferation in the form of fibrosis. Similar findings were reported by Mankin [5] in working with explants of normal human lymph nodes, in which he also found differentiation of reticulum into free forms, or macrophages, and fixed forms, or desmoblasts (fibroblasts). Others [6] have reported the formation of elastic tissue, which was not found in the present case.

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2. Maximow, A.: Bindegewebe und blutbildende Gewebe, in Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, pt. 1.

3. Erdheim, J.: Personal communication to the author.

4. Robb-Smith, A. H. T.: Beitr. z. path. Anat. u. z. allg. Path. **97**:481, 1936.

5. Mankin, Z. W.: Beitr. z. path. Anat. u. z. allg. Path. **96**:248, 1936.

6. (*a*) Klinge, F.: Deutsche Ztschr. f. Chir. **183**:195, 1923. (*b*) Wollstein, M.: Arch. Path. **12**:562, 1931.

Endothelium formed the major portion of all the neoplasms in the body. In most places the endothelial cells were normally spindle cells, while in other places they were swollen as in non-neoplastic endothelial hyperplasia. In the dural metastases they were distinctly epithelioid while in the spinal cord meningeal masses of the endothelial cells were markedly elongated and arranged in whorls, suggesting neurinoma.

The formation of forerunners of mature red and white blood cells, although not common in endothelial tumors, has been reported.[7] A cell in the neoplastic reticulum with a large nongranular body and a vacuolated nucleus with several nucleoli was also noted by Klinge,[6a] who called it a hemagonium. Similar cells are recognized in the illustrations of Kahle.[7d] Erythroblasts such as those seen in my case were described by Albrecht,[8] who had difficulty distinguishing them from the smaller tumor-lining cells in his own case. The well rounded cells lining some spaces in the cranial dural metastasis in the case I have reported might be called angioblasts. Wright [9] described the angioblast as having a shorter nucleus and more granular cytoplasm than the endothelial cell, a distinction of morphologic character rather than of cytogenesis.

Endothelium, according to Borst,[10] includes more than the cells directly lining and covering lymph and blood vessels. He would include the lining cells of the fine interfascicular spaces and the system of spaces in the connective tissue communicating with them, because it was his belief that the inflammatory and neoplastic changes of these cells are similar to those of the vascular endothelium in all respects. For Epstein [11] the problem was more complicated. He found morphologic and biologic differences between the sinus endothelial cells and the Kupffer stellate cells, on one hand, and the endothelium of the closed blood channels on the other.

Ribbert's [12] concept of endothelium, however, is still generally accepted "that only the membrane-like flat elements should be called endothelial cells which, genetically identical with the cells of the connective tissue, line the blood vessels as well as the lymph canals and lymph spaces." It is a common experience of histologists to find great

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7. (*a*) Dassel, A.: Frankfurt. Ztschr. f. Path. **36**:99, 1928. (*b*) Fischer, B.: ibid. **12**:399, 1913. (*c*) Hall, E. M.: Am. J. Path. **11**:342, 1935. (*d*) Kahle, H.: Virchows Arch. f. path. Anat. **226**:44, 1919. (*e*) Pilliet, A.: Progrès méd. **14**:50 1891. (*f*) Schlopsnies, W.: Virchows Arch. f. path. Anat. **274**:85, 1930. (*g*) Schönberg, S.: Frankfurt. Ztschr. f. Path. **79**:77, 1923. (*h*) Wollstein.[6b]

8. Albrecht, E.: München. med. Wchnschr. **49**:1135, 1902.

9. Wright, A. W.: Am. J. Path. **4**:507, 1928.

10. Borst, M.: Die Lehre von den Geschwülsten, Wiesbaden, J. F. Bergmann, 1902, vol. 1, pp. 275 and 497.

11. Epstein, E.: Med. Klin. **21**:1505, 1925.

12. Ribbert, H.: Die Geschwulstlehre für Aerzte und Studierende, ed. 2, Bonn, Friedrich Cohen, 1914, p. 200.

difficulty in distinguishing endothelial cells and fibroblasts, and, according to Maximow's [13] textbook, the differences are slight and "rapidly effaced when the endothelial cells turn into fibroblasts."

Ribbert's [12] description of endothelioma coincides with that of the manner of growth of the various tumor masses reported here. He stated that the proliferating endothelial cells "are not, or only to a slight degree and in irregular fashion, set in a closed system provided with circulating blood but [are] continually budding off and, under no functional stress, invade the surrounding tissue. The endotheliomas should grow not expansively but infiltratingly." A review of the literature indicates that relatively few cases of single endothelioma have been reported, but probably many more have been observed.[14] In these cases endothelioma was found in various organs, including cirrhotic livers.[15] Multiple endothelioma, either primary or metastatic, has been more frequently reported.[16] In so few of these cases was the growth truly malignant [17] that the question is raised as to the criteria of malignancy in endothelioma.

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13. Maximow, A.: Textbook of Histology, completed and edited by William Bloom, Philadelphia, W. B. Saunders Company, 1931, p. 85.

14. (*a*) Blumberg, A.: Virchows Arch. f. path. Anat. **261**:82, 1926. (*b*) Cannata, G. M.: Pathologica **23**:585, 1931. (*c*) Ewing, J.: Neoplastic Diseases, ed. 2, Philadelphia, W. B. Saunders Company, 1928, p. 240. (*d*) Gödel, A.: Frankfurt. Ztschr. f. Path. **29**:374, 1923. (*e*) Hedinger, E.: ibid. **3**:488, 1909. (*f*) Kothny, K.: ibid. **10**:20, 1912. (*g*) Limacher, F.: Virchows Arch. f. path. Anat. (supp.) **151**:113, 1898. (*h*) Maly, G. W.: Ztschr. f. Heilk. **19**:337, 1898. (*i*) Rintelen, F.: Klin. Monatsbl. f. Augenh. **94**:463, 1935. (*j*) Schlesinger, E.: Primäres malignes Angioendotheliom in der zirrhotischen Leber, Inaug. Dissert., Frankfurt, 1920; cited by Schönberg.[7g] (*k*) Weichselbaum, A.: Virchows Arch. f. path. Anat. **85**:554, 1881. (*l*) Fischer.[7b] (*m*) Pilliet.[7e]

15. Gödel.[14d] Kothny.[14f]

16. (*a*) de Bary, E.: Frankfurt. Ztschr. f. Path. **46**:410, 1934. (*b*) Borrmann, R.: Beitr. z. path. Anat. u. z. allg. Path. **40**:372, 1907; Verhandl. d. deutsch. path. Gesellsch. **6**:209, 1903. (*c*) Hachfeld, M.: Primärer Leberkrebs nach zirrhotischer Schrumpfung bei narbiger Obliteration der Vena cava inferior oberhalb der Leber. Primäres malignes Endotheliom der Leber, im Bilde einer Lebercirrhose, Inaug. Dissert., Halle a.S., C. A. Kaemmerer & Co., 1914; cited by Gödel.[14d] (*d*) Homans, J.: Ann. Surg. **25**:732, 1897. (*e*) Jores, L.: Zentralbl. f. allg. Path. u. path. Anat. **19**:662, 1908. (*f*) Langhans, T.: Virchows Arch. f. path. Anat. **75**:273, 1879. (*g*) Neubürger, K., and Singer, L.: Frankfurt. Ztschr. f. Path. **35**:543, 1927. (*h*) Puhr, L.: Ztschr. f. Krebsforsch. **34**:502, 1931. (*i*) Sanes, S.: Arch. Path. **22**:863, 1936. (*j*) Shennan, T.: J. Path. & Bact. **19**:139, 1914-1915. (*k*) Stamm, C.: Beitrag zur Lehre von den Blutgefässgeschwülsten, Inaug. Dissert., Göttingen, Dieterich'sche Univ.-Buchdruckerei, 1891. (*l*) Symmers, D., and Vance, M.: Am. J. M. Sc. **152**:28, 1916. (*m*) Theile: Virchows Arch. f. path. Anat. **178**:296, 1904. (*n*) Weiss, S.: Med. Klin. **7**:1273, 1911. (*o*) Klinge.[6a] (*p*) Wollstein.[6b] (*q*) Dassel.[7a] (*r*) Hall.[7c] (*s*) Kahle.[7d] (*t*) Schlopsnies.[7f] (*u*) Schönberg.[7g] (*v*) Albrecht.[8] (*w*) Wright.[9] (*x*) Ewing.[14c]

17. Schönberg.[7g] Wright.[9] Ewing.[14c] de Bary.[16a]

Ribbert,[12] as already mentioned, called particular attention to the invasive growth. Jaffé [18] recorded three features in microscopically benign endothelioma which indicate malignancy: local invasive and destructive growth, recurrence after surgical removal and production of metastases to distant organs. He also distinguishes two types: The first is the so-called cavernous hemangioma, with but little stroma and with flat lining endothelium in the blood vessels. The second type, similar to that reported here, has a cellular or syncytial stroma that may be described as embryologic. The vessels differentiate from the stroma and may become so numerous as to obscure the original nonvascular tissue, which is properly called mesenchyme. Several cases of the second type [19] have been recorded. In two of these reported cases, [20] as in the case described here, there were also marked hyperplasia and proliferation of endothelium. Von Falkowski [19a] and Klinge [6a] described the differentiation of large endothelial cells into blood cells, a feature mentioned in the case recorded here. Both types of tumors, according to Jaffé, may metastasize, while hemangioma with fully mature stroma does not. He doubts the existence of the so-called benign metastasizing hemangioma, indicating that such a tumor need not be metastatic but may originally be multiple. A review of case reports indicates that both multiple origin and metastasis may occur, and in the present case it seems probable that both did occur. One author [6b] suggested that there may be multiplicity of primary foci with malignancy expressed in local invasiveness and mitosis, while Karsner [21] conceded that there may be metastasis, as described elsewhere,[7a] if the liver contains the primary focus.

The tumor in the spleen, about which there was virtually no endothelial hyperplasia, as well as the masses in the cranial muscles invading the skull, may be considered metastatic; they contrasted strongly with those elsewhere—for example, those in the liver. In the larger tumors of the liver the endothelial tumor cells grew invasively for the most part, extending in all directions between the hyperplastic preformed sinusoidal endothelium and the hepatic cords. There were, however, much smaller neoplastic foci in the liver strongly suggesting metastasis, perhaps from hepatic foci, and the surrounding hepatic cords were compressed into slender, densely packed concentric lamellae. All in all, the tumors in the iliac fossae and perhaps the larger masses in the liver may be considered primary foci, while those in the spleen and skull may

18. Jaffé, R. H.: Arch. Path. **7**:44, 1929.

19. (*a*) von Falkowski, A.: Beitr. z. path. Anat. u. z. allg. Path. **57**:385, 1914. (*b*) Livingston, S. F., and Klemperer, P.: Arch. Path. **1**:899, 1926. (*c*) Klinge.[6a] (*d*) Ewing.[14c] (*e*) Borrmann.[16b] (*f*) Shennan.[16j] (*g*) Stamm.[16k]

20. Shennan.[16j] Stamm.[16k]

21. Karsner, H. T.: Human Pathology, ed. 4, Philadelphia, J. B. Lippincott Company, 1935, p. 386.

be considered as metastases. Because of the not infrequent occurrence of primary vascular tumors in the vertebral column,[22] those in the case described are probably to be considered as primary.

Single endothelioma has been called by some hamartoma,[23] and multiple endothelioma, systemic hamartoma.[7f] Others regard multiplicity as evidence of a systemic endothelial disease.[24] It is noteworthy that in the case which I have reported the lymph nodes were free from change. Fischer [7b] in his case of hepatic endothelioma with blood cell formation thought, like Ribbert,[12] who commented on the case, that "cellular malformation [is] . . . the basis of the tumor's origin," an embryonal defect of the anlage of the entire capillary endothelium of the liver, with the entire organ the primary focus. One cannot determine from Fischer's illustrations whether tumor invasion between preexistent capillary endothelium and hepatic cords was ruled out. In the liver in the present case only careful study revealed that invasion, not endothelial transformation to neoplasm, was responsible for the growth of the tumor.

Albrecht [8] reporting a case of "hemangio-endothelioma" of the dura metastasizing to the urinary bladder thought the cause to be a displacement of a focus of vasoformative cells of the mesenchymoblast of the meninges or a return to the mother cell type. Livingston and Klemperer [19b] and Klinge [6a] regarded their own as well as Borrmann's [16b] case as one of unripe mesenchymal blastoma with predominance of angiomatous structures. Borst [25] believed that the tumor in his case arose in indifferent mesenchymal syncytium, as did von Falkowski.[19a] For the latter the neoplasm began in those areas of the organ where the parenchyma did not develop because of lower potentiality of mesenchymal differentiation.

A scale of differentiation of endothelial tumors was offered by Klinge,[6a] based on his own case of "malignant mesenchymal angioma" of the finger. The first step is in the earliest masses in skin, muscles and lungs, formed of a mesenchymal syncytium with all signs of malignancy. These signs include regional invasion and rapid unicentric enlargement of the growth within a few weeks. In a later stage spaces appear, lined by from one to several layers of epithelioid cells, which produce blood cells. The mesenchyme between the irregular capillaries has now differentiated into true blood-forming cells, suggesting red

22. Behrmann, A.: Haemangiome ungewöhnlicher Lokalisation, Inaug. Dissert., Göttingen, Höxter a. d. Weser, C. B. Flotho, 1927.

23. Pilliet.[7e] Schönberg.[7g] Rintelen.[14i]

24. Grabowski, W.: Arb. a. d. path. anat. Inst. d. Univ. Polens **2**:1, 1927; cited by Dassel.[7a] Goldschmid, E., and Isaac, S.: Deutsches Arch. f. klin. Med. **138**: 291, 1921-1922. Neubürger and Singer.[16g] Puhr.[16h]

25. Borst, M.: Geschwülste des Gefässgewebe, in Aschoff, L.: Pathologische Anatomie, ed. 7, Jena, Gustav Fischer, 1928, vol. 1, p. 729.

marrow. As the blood nodes develop, there are fewer strands of mesenchymal tissue, and capillaries tend to disappear. This is the third stage. In the next stage the strands and capillaries have disappeared, and there are left only endothelium-lined spaces filled with blood that empties into neighboring blood vessels. The highest level is that of cavernous spaces which have exhausted the hematoblastic potential. A similar concept was held by Livingston and Klemperer.[19b]

Kahle [7d] believed that cells other than the Kupffer stellate cells were the mother cells of the hemoblastic tumor in the liver. He contended that there was much in support of Löhlein's [26] view that the primary process is a failure of the hepatic cells and that the change in the blood vessel apparatus is only secondary. Further growth is achieved by breaking into the branches of the larger vessels, the tumor emboli traveling in the small portal radicals. Kahle admitted, however, that the changes in the large vessels might be the original process. Schlesinger [14j] assumed an embryonal developmental disturbance, with resultant disease of the capillary system of the liver. He made no attempt to explain the significance of the concomitant cirrhosis of the liver, nor did Kothny [14f] in a similar case.

The nomenclature has varied greatly. "Angioma" should be reserved for the most mature type, in which the complete vessel, including smooth muscle, is the basic unit. The presence in different parts of the body of mature benign angioma is best explained by a multiple origin, and the condition called multiple angioma. For the less mature forms Kothny [14f] proposed "hemangio-endothelioma" or "hemangio-endotheliosarcoma" because of the "sarcomatous" character and the resemblance of the Kupffer cells in his case to spindle cells. Twenty years later, Erdheim,[3] Kothny's mentor, wrote that he disliked the name "angiosarcoma," without, however, giving his reasons. Rintelen [14i] suggested "hemangiosarcoma (lymphangiosarcoma) endotheliomatosum." The neoplasms so designated are in turn subdivided into homotypic and heterotypic forms. The homotypic are: (1) angioma simplex, which includes a few small arteries and veins, (2) angioma cavernosum and (3) angioma endotheliomatosum. Under the heterotypic form Rintelen places malignant variations of the homotypic.

Schlopsnies [7f] thought the name first proposed by Borst,[10] "angioplastic sarcoma," appropriate, while Theile [16m] suggested "sarcomatous angioma." Borrmann [16b] rejected "sarcoma," since it applies to stroma. Later Borst [25] reserved "angiosarcoma" for tumors arising from the endothelium of the perivascular lymph space, or so-called perithelium, and "angioma sarcomatosum" for tumors like that with which this paper is concerned.

It is suggested that "hemendothelioma" is a satisfactory name, as well as "lymphendothelioma," when endothelium is the chief tissue in

26. Löhlein, M.: Verhandl. d. deutsch. path. Gesellsch. **13**:320, 1919.

the neoplasm. When the criteria of malignancy given by Jaffé [18] are satisfied, "malignant hemendothelioma" may be used, whether the growth is single, multiple or metastatic. If, as in the present case, the potentiality of the mesenchyme is still present, this term may be qualified by the adjective "mesenchymal."

The possible relationship between the neoplasm reported here and Kaposi's disease (sarcoma idiopathicum haemorrhagicum), in which there are multiple vascular foci, is of interest, especially since one authority [27] who had seen nonosseous sections of the neoplasm reported here "favored the diagnosis of hemangioendothelioblastoma, probably of the Kaposi type." In that disease the symmetry of the distribution and localization of lesions at the ends of the extremities is characteristic, although lesions are also formed elsewhere in the body. In endothelioma there is no predilection as to sex,[22] while in Kaposi's disease the patients are mostly men.[28] Of thirty-four cases reviewed for this study in which the sex was stated, twenty were observed in females and fourteen in males. In Kaposi's disease nodules looking like cavernoma are seen in the liver. Foci are rarely found in the spleen, whereas that organ is not an uncommon site for the vascular tumor as reported in the literature.

Histologically the principal features are new formation and ectasia of the capillaries, chiefly of the blood vessels, and connective tissue proliferation. Pigmentation is secondary to hemorrhage. There are reactive processes in vessels and connective tissue and finally signs of regression and healing with scarlike tissue. The picture varies with predominance of vessel or of connective tissue changes and with the circumscribed or diffuse character of these changes.

The spindle cells, so prominent a feature of the focus of Kaposi's disease, are of disputed character. Figure 29 of Kren's monograph [28] shows spindle cells with partly rounded nuclei similar to those seen in the dural metastases of the case reported here. He wrote that "one can no longer distinguish tumor cell from [blood vessel] wall cell. Only where a dilated capillary is bordered on one side by the tumor mass and on the other by a slender connective tissue mass can one determine that the vessel on the connective tissue side really has an endothelial lining; so much do endothelial cell and tumor cell appear identical."

Sternberg [29] asserted that the spindle cells are smooth muscle cells and that Kaposi's disease is hamartomatous. The spindle cells, although like muscle cells in form, do not stain similarly. Schaffer [29] was unable to determine definitely whether the cells had the character of connective

27. Mallory, F. B.: Personal communication to the author.

28. Kren, O.: Sarcoma idiopathicum hemorrhagicum (Kaposi), in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1933, vol. 12, pt. 3.

29. Quoted by Kren.[28]

tissue or of muscle. Kren bridged the difficulty by writing that "the little we definitely know is that they are cells of the mesoderm." He quoted Ewing that the disease is a hamartoma in that embryonal cells begin to proliferate under the same stimulus so that there is primary multiple tumor formation.

There are other indications that endothelioma and Kaposi's disease may have elements in common since Kren wrote that "the pathogenesis of the Kaposi sarcoma . . . might be conceived as an analog of the similar process in the liver (hemangio-endothelioma). This concept would also explain the so-called metastases, the recurrences in the involved areas and other features. It would mean a *systemic disease of the reticulo-endothelial apparatus* of the skin, in harmony with which one could bring in the fact that in most cases of Kaposi sarcoma monocytosis can be observed. That concept rests on the fact that monocytes arise from the reticulo-endothelial system."

NOTE.—Bevacqua's [30] case is not included in this study because, as Ribbert [12] wrote, it "gives the impression of epithelial, that is, metastatic tumors. The necropsy was only incompletely performed." The cases of Carstenson [31] and Chutro [32] are instances of glomus tumors. Other cases are rejected because the descriptions suggest angioma [33] or telangiectatic osteogenic sarcoma.[34]

SUMMARY

A 65 year old man was in the hospital for six weeks before he died of bronchopneumonia. Three days before admission, after several months of generalized aches and pains, complete paralysis appeared below the level of the second thoracic vertebra. At necropsy there were numerous vascular neoplasms throughout the body—in viscera, soft tissues and skeleton. The largest (18 by 13 by 10 cm., weighing 1,170 Gm.) was in the right iliac fossa. The lymph nodes were not involved.

Microscopically the tumors were basically similar. A primitive endothelial syncytium formed the chief tissue, with areas strongly suggestive of mesenchyme. Bone formation was seen in the largest new growth, where hematopoiesis was most marked. Collagen fibers were formed in some places, but building of elastic tissue was not noted. It was thought that the multiplicity of tumors was due in part to multiplicity of primary foci and in part to metastasis. The nomenclature and similarities to Kaposi's disease are discussed.

30. Bevacqua, A.: Virchows Arch. f. path. Anat. **200**:101, 1910.

31. Carstenson, I.: Arch. f. klin. Chir. **144**:409, 1927.

32. Chutro, P.: Angioma subungeal. Glomus? Angiosarcoma? in Livre jubilaire offert au professeur H. Hartmann par ses amis et élèves, Paris, Masson & Cie, 1932, p. 111.

33. Koch, I.: Arch. f. klin. Chir. **153**:170, 1928. Roggenbau, F.: Beitr. z. path. Anat. u. z. allg. Path. **49**:313, 1910.

34. Markowitz, B.: Am. J. Clin. Path. **5**:333, 1935.

REACTION OF LYMPHATIC TISSUE IN EARLY STAGES OF BACTERIUM MONOCYTOGENES INFECTION

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The isolation of Bacterium monocytogenes by Murray, Webb and Swann [1] promised to be of great aid in clearing up much of the controversy which has dominated the question of the origin of the monocytes of the blood, for when appropriate doses of a culture of this bacterium are injected into rabbits, monocytosis ensues in the blood stream with great regularity. In view of this it seems that a careful histologic analysis of the organs and viscera of these rabbits should disclose the site of origin and the cells from which the monocytes develop. But this has not been the case, for the several investigators who have used this bacterium to study the precursor of the monocyte offer divergent views on the origin of this cell (Murray, Webb and Swann;[1] Bloom;[2] Lang;[3] Nyfeldt;[4] Rezzesi;[5] Wallbach;[6] Levi and Penati[7]). The details of their reports are considered in this paper in the comment; here it is only necessary to point out that all of these investigators failed to examine the tissues of their infected animals before the number of monocytes in the circulating blood had increased.

In an attempt to determine the source of the monocytosis and to explain some of the conflicting reports on the effects of the injection of this bacterium, I have studied the blood and organs of a series of rabbits which were infected with portions of the same cultures of the

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1. Murray, E. D. G.; Webb, R. A., and Swann, M. B. R.: J. Path. & Bact. **29**:407, 1926.

2. Bloom, W.: Folia haemat. **37**:1, 1928.

3. Lang, F. J.: Folia haemat. **36**:383, 1928.

4. Nyfeldt, A.: Folia haemat. **47**:1, 1932.

5. Rezzesi, F. D.: Haematologica **14**:239 and 287, 1933.

6. Wallbach, G.: Arch. d'anat. micr. **30**:275, 1934.

7. (*a*) Levi, G. M., and Penati, F.: Arch. per le sc. med. **58**:773, 1934; (*b*) Haematologica **16**:317, 1935. (*c*) Penati, F., and Levi, G. M.: ibid. **16**:261 and 409, 1935.

bacterium and which were killed at close intervals in the period from the time of injection until the monocytes were present in the blood in great numbers.

One cannot tell the degree of virulence of this bacterium as it was used by most of the previous investigators. Indeed, there is evidence in their reports that during the course of their experiments the organism showed marked variations in virulence. In order to have the virulence of the bacterium in this and in other studies constant, I decided to use as the criterion of virulence the dosage of bacteria necessary to produce a given degree of monocytosis in the peripheral blood in forty-eight hours. This work was extended to a larger series of guinea-pigs to see if this species also reacts to infection with this bacterium with monocytosis, as Murray, Webb and Swann intimated. Another reason for infecting guinea-pigs was to see the effect of this infection on the Kurloff bodies of the lymphocytes and monocytes.

There is no difficulty in distinguishing the typical monocyte of the blood from cells of all other types with the possible exception of those in Naegeli's *monocytoide Myeloblastenleukämie* and occasional nongranular cells (lymphocytes) of normal blood. This separation can be carried out as easily with unstained cells as in supravital, dry or wet smear preparations or on well stained sections. Unfortunately the precursor of the monocyte lacks morphologic criteria by which it can be separated from the precursors of the granulocytes and from the lymphocytes. The literature contains many claims of the morphologic distinction of "monoblasts" from other stem cells. But the multiplicity of the criteria offered by the different investigators is in itself evidence of the insufficiency of most and, as I believe, of all of them.

In view of the conflicting nomenclatures which have been applied to the constituents of the lymphatic tissue in the lymph nodes and in the spleen it is necessary to define the terms which will be used here. The *lymphatic nodule* is a globular accumulation of lymphocytes within dense or loose, diffuse lymphatic tissue. It may be composed mainly of cells of the same type throughout or it may show variations in structure in its different portions. Sometimes its center may consist mainly of reticular cells or macrophages and few lymphocytes or of fibroblast-like cells; this is a *reaction* center. At other times its central portion may be a focus of rapidly multiplying medium-sized and large lymphocytes; this is a *lymphocytopoietic* center. As will be pointed out later, this focus of proliferating lymphocytes may or may not be surrounded by a limiting zone of smaller, inactive lymphocytes. A more extended discussion of these terms and the necessity for using them will be found elsewhere (Conway [8]).

8. Conway, E. A.: Anat. Rec. **69**:487, 1937.

The conflicting opinions on the meaning of the term "malpighian corpuscle" are due to the fact that this name was given to the white pulp of the spleen as seen macroscopically or with low magnification. Some microscopists now use the term to include the white pulp, i. e., the entire periarterial lymphatic tissue sheath, a view to which I would subscribe but for the fact that most pathologists use the term only for the swellings of the sheath caused by the presence of lymphatic nodules within it. On the other hand there are some who describe and picture the sheath of lymphatic tissue with its enclosed artery in cross-section as the "malpighian corpuscle." It is obvious that in a longitudinal section of the artery and its lymphatic tissue sheath at most two nodules can be seen at any point in the sheath, i. e., one on each side of the artery. In cross-section, however, three or more nodules, if present, can be seen in the sheath. Again, if only one nodule is present in the lymphatic tissue of the sheath at a given point, a cross-section here shows the artery to be eccentric in the sheath in most cases. This is the usual description of the "malpighian corpuscle." In cross-section through a part of the sheath in which no nodules are present the artery is centrally located. Finally, the "central" artery sometimes passes through the center of a nodule.

Accordingly, it seems best to dispense with the term "malpighian corpuscle" until there is a consensus as to its meaning. I shall therefore speak of the white pulp of the spleen as the periarterial sheath of lymphatic tissue and its contained nodules as seen in longitudinal sections and shall specifically designate the description of a region of the sheath which happens to be seen in cross-section.

This report covers only the changes in the peripheral blood, the mesenteric and cervical lymph nodes and the spleens of rabbits and guinea-pigs infected with a single standardized dose of Bact. monocytogenes. The histologic changes in omentum, liver, bone marrow, thymus, thyroid, lung, heart, adrenal and kidney were found to have no direct bearing on the origin of the monocytogenic response and are reported elsewhere. The histologic changes in reinfection of rabbits immunized to Bact. monocytogenes will also be included in another report.

This work was carried out at the suggestion of Dr. W. Bloom.

MATERIAL AND METHODS

The strain of Bact. monocytogenes used in these experiments was strain 58, which was furnished by Dr. E. G. D. Murray. It had not been subjected to animal passage for two years previous to this use and had the morphologic characteristics of the avirulent form; i. e., the culture showed long filamentous rods. This avirulent culture served as the stock strain from which both the strain virulent for rabbits and that virulent for guinea-pigs were obtained.

Three successive passages in young adult rabbits sufficed to raise the virulence to the desired degree; 0.2 cc. of a suspension of the growth of three twenty-four hour dextrose-veal infusion agar slants (p_H 7.2) when injected intravenously into a rabbit produced on the average a monocytosis equivalent to 25 per cent of the total number of leukocytes in the peripheral blood forty-eight hours after the injection. As all of the rabbits showed about the same leukocyte count at similar intervals after the injection of the standard dose, the criterion of the "percentage of monocytes" suffices. The injection of this standardized dose did not kill any of the test rabbits in five days.

The ten rabbits of the experimental series included in this report were all young adult males. They were all subjected to injection on the same day; each received 0.2 cc. of the same bacterial suspension, prepared as described. The injection was made intravenously in the marginal vein of the ear. The rabbits were killed one, three, six, nine, twelve, eighteen, twenty-four, thirty-six, forty-eight and sixty-six hours after the injection. Two uninfected rabbits were killed on the same day as the sixty-six hour infected rabbit.

Another portion of the avirulent stock strain of Bact. monocytogenes was subjected to five successive passages in guinea-pigs weighing about 300 Gm. each. The virulence of Bact. monocytogenes recovered from the spleen and liver of the fifth passage guinea-pig was determined as in the case of the rabbit. In this instance 0.1 cc. of a suspension made from the growth of two twenty-four hour dextrose-veal infusion agar slants when injected intravenously into a guinea-pig also produced on the average a monocytosis equivalent to 25 per cent of the total number of leukocytes in the peripheral blood in forty-eight hours. This standardized dose did not kill any test guinea-pig in ten days. In preliminary experiments it was found that with five times the dose used here the same process occurred as will be described for these guinea-pigs, but the entire response was more intense and more rapid and killed the animals in forty-eight hours.

The sixteen guinea-pigs of the experimental series included in this report were male guinea-pigs weighing approximately 300 Gm. each. As in the case of the rabbits, they were all submitted to injection on the same day, each receiving 0.1 cc. of the same suspension of Bact. monocytogenes, prepared as described. The injection was given in the femoral vein, and the guinea-pigs were killed one, three, six, nine, twelve, eighteen, twenty-four, thirty-six, forty-three and forty-eight hours and three, three and a half, four, five, six and seven days after the injection. One control guinea-pig was killed on the day the injection was made and two on the day the last of the test animals was killed. The series of guinea-pigs used was larger than that of rabbits, because the later stages of the infection in rabbits have been described repeatedly, while the course and histologic picture of experimental infection in guinea-pigs do not seem to have been studied.

White blood counts and wet and dry fixed smears were made from blood taken from the ear vein of each animal just prior to injection and again just before killing. In preliminary experiments which were made to determine the virulence of the bacterium, supravital preparations were made of the peripheral blood and of the hematopoietic organs in addition to the dry and moist fixed smears. But it was soon found that the supravital smears added little to the clearcut information obtained from the moist and dry fixed blood and organ smears and from the sections of the organs. Accordingly, as the latter methods formed a permanent record of the experiment and gave as much information as the supravital films, the supravital method was discarded in this series. In the rabbit series blood was also taken to determine the preinjection titer of agglutinins to Bact. monocytogenes, if any were present. The tissues were removed from all the animals while they

were under ether anesthesia, fixed immediately in Zenker-Helly-Maximow solution, embedded in pyroxylin (nitrocellulose), serially sectioned at 8 microns and stained with hematoxylin-eosin-azure II and by the short Foot method for silver impregnation of reticular fibers. Some of the tissues of each organ removed from the rabbits were also fixed in absolute alcohol for basophil leukocytes. The granules of the basophil leukocytes of the guinea-pig are present in material fixed in Zenker-Helly-Maximow solution and stained with hematoxylin-eosin-azure II.

OBSERVATIONS ON THE PERIPHERAL BLOOD OF INFECTED GUINEA-PIGS

The average white blood cell count before the injection was 6,100 cells per cubic millimeter; the highest count was 7,200 and the lowest 5,400. In the animals killed during the first nine hours following the injection there was marked fluctuation in the white cell count. Then followed progressive leukopenia, reaching 4,100 at thirty-six hours, a fairly normal count, 7,300 at forty-three hours and 6,400 at forty-eight hours, leukopenia, 3,900, during the third day, and an increase to 7,800 on the fourth day. From this time on the white count increased progressively to the seventh day, when it reached 10,650 cells per cubic millimeter.

The monocytes began to increase appreciably at twelve hours, when they were 13 per cent of the total number of leukocytes of the guinea-pig, compared with an average of 3 per cent before the injection. At forty-eight hours they numbered 26 per cent of the total number of leukocytes, while at three and a half days they constituted 40 per cent. Then they decreased progressively to 15 per cent on the seventh day.

The large lymphocytes increased from 3 per cent during the first nine hours to 18 per cent at twenty-four hours, with subsequent oscillations between 12 and 20 per cent during the remainder of the experiment. The number of small lymphocytes rose sharply from a preinjection average of 26 per cent to forty-eight per cent in twenty-four hours, which was followed by a progressive decrease as the monocytes increased; the small lymphocytes decreased to 9 per cent on the third day, during which time the monocytes constituted 40 per cent of the total number of leukocytes.

The average number of heterophil (pseudo-eosinophil) leukocytes before the injection was 68 per cent of the total number of leukocytes. They decreased from 70 per cent at nine hours to 18 per cent at twenty-four hours. There was then a gradual but progressive increase to 52 per cent three days after the injection, that level being maintained to the seventh day. At twenty-four hours the basophil leukocytes had increased to 9 per cent of the total number of leukocytes in the peripheral blood, an average of less than 1 per cent having been present before the injection.

There was no marked change in the number of the Kurloff bodies in the various-sized lymphocytes and monocytes in the smears of the peripheral blood.

GROSS ANATOMIC OBSERVATIONS ON INFECTED GUINEA-PIGS

There was a large amount of peritoneal fluid in the guinea-pigs killed forty-three and forty-eight hours after the injection. The cervical lymph nodes in these animals showed evidence of hemorrhage. The guinea-pigs killed at forty-eight hours had very small mesenteric lymph nodes; in those killed at three days the corresponding nodes were so small that it was difficult to obtain a piece for section. In the animal killed four days after the injection, the mesenteric lymph node was

larger than normal, and increases in size were shown with each successive day, that in the guinea-pig killed seven days after the injection being 3.5 cm. in its longest dimension. In this guinea-pig, in a small portion of the mesentery the lymphatic vessels were swollen and were separated by small white nodules which in sections were found to be developing lymph nodes.

MICROSCOPIC OBSERVATIONS ON INFECTED GUINEA-PIGS

Spleen.—In the spleen of the control uninfected guinea-pig the white pulp appeared as diffuse lymphatic tissue which formed a sheath for the branches of the arteries and contained rather ill-defined lymphatic nodules. Pale-staining central areas were present in some; a few of these were typical reaction centers, with prominent reticular cells, free macrophages and some cellular débris, while others consisted mainly of medium lymphocytes. The sinuses of the red pulp contained a moderate number of small lymphocytes, free macrophages and erythrocytes. There were also some heterophil leukocytes and an occasional monocyte. The nuclei of the reticular cells were prominent in the Billroth cords. The free cells here were mainly small and medium lymphocytes and heterophil leukocytes.

One hour after injection of the infecting dose of Bact. monocytogenes, practically all of the nodules and most of the lymphatic tissue sheaths had more or less well defined margins of medium lymphocytes. Within these margins the predominant free cell was the small lymphocyte. In some of the lymphatic tissue sheaths there were isolated, well demarcated compact accumulations of medium lymphocytes. The red pulp near the margins of the sheaths contained many more medium-sized lymphocytes than were seen in the control spleens. There were increased numbers of heterophil leukocytes in both the sinuses and the cords. The reticular cells throughout the red pulp showed no change and no mitoses.

At three hours, the margins of the lymphatic tissue sheaths, which had been sharply demarcated, were spreading out into the red pulp. Most of the white pulp was less dense than at the one hour stage, and its reticular cells were more prominent. Throughout the lymphatic tissue sheaths there were a number of lymphocytopoietic centers in the nodules. Many of these in turn had reaction centers within them. The cross-section of one lymphatic tissue sheath contained two nodules with lymphocytopoietic centers. Accumulations of plasma cells and lymphocytes were prominent around the trabeculae. The lymphocytes containing Kurloff bodies were increased in number in the sinuses and cords, and the latter contained great numbers of plasma cells. The reticular cells of the cords showed no change.

At six hours, the periarterial lymphatic tissue sheaths in most places were very thin and quite sharply demarcated from the red pulp. The nodules were smaller and contained small lymphocytes centrally; some of the nodules were surrounded by a zone of medium-sized lymphocytes; others, by more densely packed small lymphocytes. There were only a few small areas of active proliferation of lymphocytes in the diffuse lymphatic tissue of the periarterial sheaths.

The sinuses had many more cells than at the previous stage; the heterophil leukocytes were increased in number, and there were a few circulating myelocytes and an increased number of monocytes. The majority of the Kurloff bodies were in medium-sized and small lymphocytes. There were some large basophil lymphocytes in the pulp cords; many of them were ameboid. One reticular cell in the red pulp was in mitosis. A few basophil myelocytes and mature basophil leukocytes were present in the cords.

At nine hours, the lymphatic tissue sheaths were still narrow, and their margins merged gradually into the red pulp. Most of the nodules were larger and were surrounded by zones of medium-sized and small lymphocytes, with an occasional large lymphocyte among them. Some of the arterioles of the red plup were surrounded by zones of medium and small lymphocytes. The number of basophil leukocytes in the red pulp was increased and a few eosinophil leukocytes were present. There were only a few Kurloff bodies in the many lymphocytes of the red pulp. The reticular cells of the red pulp showed no change.

At twelve hours, the sinuses were crowded with lymphocytes of all sizes; some of these were medium-sized lymphocytes, many of which were in mitosis. There was also an increase in the number of large basophil lymphocytes in the cords and sinuses. The lymphatic tissue sheaths were much narrower than in the previous animals, but the nodules were more numerous than at nine hours. From the borders of the sheaths irregular areas of medium lymphocytes extended far into the red pulp; in these areas were many ameboid lymphocytes. Many of the larger nodules had reaction centers; a few had lymphocytopoietic centers. Despite the crowding of the sinuses and pulp cords with lymphocytes, the reticular cells were still distinct, and at this time no mitoses were found in them.

At eighteen hours the periarterial lymphatic tissue was much more extensive than at any previous stage, and its margins extended further into the red pulp. These lymphatic tissue sheaths were wide, and each along practically its entire course had an inner zone of predominantly small lymphocytes and fairly prominent reticular cells and an outer denser zone of medium-sized and larger lymphocytes. This outer zone was not sharply circumscribed and merged into the red pulp. Here and there along the sheaths were many small nodules of proliferating lymphocytes. Some of the sheaths in cross-section were quite large. One had four nodules consisting of proliferating medium-sized lymphocytes, while some sheaths had two or three such areas. Some nodules in the cross-section had an outer zone of medium-sized lymphocytes, while others consisted of densely packed medium-sized lymphocytes, many of which were in mitosis, some larger lymphocytes and a few prominent reticular cells.

Large basophil lymphocytes were very numerous in the red pulp, and many of these were in mitosis. In areas adjacent to the margins of the sheaths it was very easy to see that these large lymphocytes had moved into the red pulp from the lymphatic tissue of the sheaths. There were many ameboid lymphocytes in these regions of the red pulp. There was no evidence of the multiplication of the reticular cells of the red pulp or of their transformation into the numerous medium-sized and large lymphocytes that crowded the red pulp. There were many basophil leukocytes in the sinuses, and the monocytes were more numerous here than at twelve hours. Numerous areas of plasma cells were also to be found throughout the red pulp.

At twenty-four hours, the periarterial lymphatic tissue sheaths were very large. This white pulp extended far into the cords of Billroth. Many of the lymphocytopoietic centers of the nodules had paler-staining central areas of prominent reticular cells and a few macrophages; these were beginning reaction centers. At the same time cross-sections of several of the lymphatic tissue sheaths showed four or five lymphocytopoietic foci. Several of the larger foci were somewhat circumscribed by marginal zones of smaller lymphocytes, but two smaller ones in particular were areas of lymphocyte proliferation beginning in the diffuse lymphatic tissue of the sheath independently of a preexisting nodule.

The predominant free cell in the red pulp was a large basophil lymphocyte. Many of the large basophil lymphocytes in the cords were dividing. The reticular

cells here were quiescent and showed no evidence of proliferation. The sinuses contained many monocytoid cells. There were only a few Kurloff bodies, and the number of plasma cells was markedly increased. The spleen up to this time had reacted to the infection with a marked increase in the number and size of the lymphocytes in the red pulp; these lymphocytes for the most part had migrated here after their extensive multiplication in the lymphatic nodules and, to a somewhat lesser extent, in the diffuse lymphatic tissue of the periarterial sheaths. This formation and migration were so great that in many places the margins of the white pulp had extended far into the red pulp; in such areas only the sinuses distinguished the former red pulp from the white.

At thirty-six hours, there was a distinct change in the cellular content of the sinuses and cords. The majority of the lymphocytes were smaller and less basophil; many of them had the appearance of monocytoid lymphocytes. The large nodules were still numerous in the sheaths, although they were less frequent than at twenty-four hours; there were a very few small ones at this time. The margins of the periarterial lymphatic tissue sheaths and, in places, of their larger nodules extended far out into the red pulp. At this time the outer zone of both the nodular and the diffuse lymphatic tissue consisted mainly of monocytes and monocytoid lymphocytes.

The term "monocytoid lymphocytes" includes intermediate cells which cannot be classified as lymphocytes or as monocytes. This group includes many cells whose nuclei have the characteristic large clumps of chromatin, one or more distinct, fairly acidophil nucleoli and the rather thick nuclear membrane of the lymphocyte. The cytoplasm of these cells is more abundant and in some cases paler-staining than that of the typical small and medium-sized lymphocytes. In other cells the nuclei are somewhat larger and more irregular in outline, and the chromatin particles are less clumped than in the lymphocytes, while the nucleoli are smaller and more numerous.

Many of the centers of the lymphatic nodules were pale-staining because of their conspicuous reticular cells. Often some of these cells were just retracting their cytoplasm and becoming basophil. While many of the centers of the nodules were exhausted and practically devoid of lymphocytes, in others there were a few lymphocytes in mitosis. In some of the nodules there were monocytoid lymphocytes and a few monocytes. These monocytes were easily distinguished from the adjacent lymphocytes by their indented nuclei with one or more acidophil nucleoli. The cytoplasm of these cells was abundant in comparison with the small size of the nucleus and stained a pale blue.

At forty-three hours the relative amount of white pulp, as well as its lymphocytopoietic activity, was considerably less than at thirty-six hours. The periarterial sheaths were almost depleted of larger lymphocytes, the cells being mainly small lymphocytes and numerous monocytoid lymphocytes. The nodules were small and far less numerous than at thirty-six hours. In cross-section the lymphatic sheaths appeared small, for the most part. Several larger sheaths in cross-section had medium and a few large lymphocytes on one side and monocytoid lymphocytes and monocytes on the other. A few of the larger nodules appeared to be almost entirely monocytic.

The peritrabecular areas contained more medium-sized and small lymphocytes and fewer plasma cells than previously. Throughout the cell-crowded sinuses and

pulp cords it was very difficult to classify all of the medium-sized cells as either lymphocytes or monocytes. The sinuses were filled with all transition stages from various-sized lymphocytes to monocytes. The reticular cells of the pulp and the lining cells of the sinuses showed no change. There was no evidence of their transformation into monocytes at this or previous stages.

At forty-eight hours, the periarterial lymphatic tissue sheaths had practically disappeared except for a few scattered large nodules, some of which had centers of proliferating lymphocytes. There were many more monocytes and monocytoid lymphocytes throughout the spleen (fig. 1 *B*). As in all previous stages the reticular cells were quiescent and showed no evidence of change. Groups of plasma cells were again prominent, especially around the trabeculae.

At three days, the cords and sinuses of the red pulp were full of monocytes and monocytoid lymphocytes. Many lymphoid cells in the red pulp were in mitosis. The periarterial lymphatic tissue sheaths were slightly more prominent than at forty-eight hours. But instead of the free cells being mainly lymphocytes as in the normal animal, there were monocytoid lymphocytes and monocytes in great numbers in both the diffuse tissue and the nodules of the lymphatic sheaths. Along these sheaths were scattered areas of lymphocytic proliferation, but even in these areas one could find monocytes. There was no evidence of changes in the reticular cells of the red pulp. There were some very large lymphocytes here and there throughout the section. The foci of plasma cells were few and small.

At three and a half days, the periarterial lymphatic tissue sheaths were considerably more extensive than during the previous twenty-four hours; the margins were not sharply delimited from the red pulp. The nodules were larger and somewhat more numerous than at three days. There was an extensive infiltration of actively ameboid lymphocytes of all sizes into the cords and sinuses of the red pulp adjacent to these spreading margins of the sheaths. The nodules and the diffuse lymphatic tissue of the sheaths contained many large lymphocytes; some of these were in mitosis. The entire white pulp appeared diffusely lymphocytopoietic, although this process in a few places was accentuated in foci of proliferating lymphocytes. There were no mitoses in the reticular cells of the red pulp or evidence of their change into lymphocytes, although many of the lymphocytes in these areas were actively multiplying. The cords and sinuses of the red pulp were crowded with lymphocytes which had migrated into the red pulp from the white pulp. The number of monocytes was still high in the cords and sinuses but had decreased appreciably in the lymphatic tissue of the arterial sheaths. The regions around some of the trabeculae appeared as diffuse lymphatic tissue; plasma cells were very infrequent in these areas.

At four days, the increase in the lymphatic tissue around the arteries was still more marked than at the previous stage. The numerous nodular areas of proliferating lymphocytes throughout it varied greatly in size as well as in structure. Some of these lymphocytopoietic foci were sharply demarcated by smaller lymphocytes; others had no delimiting corona of densely packed cells, while in some there were three or four concentric zones of lymphocytes of different sizes, showing evidence of several different cycles of formation of lymphocytes in one nodule. The cross-section of one sheath showed three centers of proliferating lymphocytes in one lymphatic nodule. The outer zones of many of the nodules contained exceedingly numerous monocytes. The cords and siuses contained many monocytes and monocytoid lymphocytes; the heterophil leukocytes were abundant throughout the red pulp.

At five days, the medium-sized and large lymphocytes were more numerous throughout the entire spleen, but especially in the lymphatic tissue sheaths, than

at any previous hour. The lymphatic nodules in the sheaths were very large and contained many proliferating medium-sized and large lymphocytes. The reticular cells of the red pulp appeared normal. The cords and sinuses still contained many monocytes and all stages of monocytoid lymphocytes. Heterophil leukocytes were still very numerous in the red pulp, and there were a few circulating myelocytes and megakaryocytes.

At six days, the cords and sinuses were crowded with lymphocytes of all sizes, monocytes, monocytoid lymphocytes and many heterophil leukocytes; erythrophagocytosis by macrophages was marked. The reticular cells of the red pulp appeared inactive. Plasma cells were very numerous in both cords and sinuses.

The periarterial lymphatic tissue of the sheaths was extensive, and throughout it were many lymphatic nodules. The margins of some of these were sharp; others had many ameboid cells migrating out from them. In a few nodules paler-staining reaction centers had appeared, while in others the pale-staining central portions consisted mainly of very large lymphocytes. There were only a few monocytes in the white pulp.

At seven days, the sinuses of the red pulp were crowded with small and medium-sized lymphocytes, monocytes and monocytoid lymphocytes. There were some macrophages and heterophil leukocytes and some small lymphocytes with Kurloff bodies. The increase in the lymphatic tissue of the periarterial sheaths was even greater than at six days, and many of the nodules showed three or four different cycles. In these the centrally located medium lymphocytes were surrounded by a ring of small lymphocytes, and these in turn by an external zone of medium-sized and large lymphocytes; while in others the central area was made up of large basophil lymphocytes, with some in mitosis, and around these was a zone of medium lymphocytes, with many in mitosis. The latter in turn were surrounded by one or more zones of small and large lymphocytes.

Summary.—The first reaction in the spleen of the guinea-pig to the injection of Bact. monocytogenes is a movement of lymphocytes from the lymphatic tissue of the periarterial sheaths into the red pulp. This loss of lymphocytes by the lymphatic tissue is quickly compensated by an intensive new formation of lymphocytes in these sheaths. During the first twenty-four hours of the infection more lymphocytes are formed here than are lost. The extensive migration of lymphocytes from the sheaths is the source of the many large and medium lymphocytes that crowd the red pulp at twenty-four hours. These lymphocytes are connected by an intimate series of transition forms (monocytoid lymphocytes) to the many monocytes present in the red pulp. These transitions are more frequent during the second than during the first day. At thirty-six and forty-eight hours and during the third day these monocytoid lymphocytes, as well as recognizable monocytes, are present not only in the red pulp but also in the diffuse and nodular lymphatic tissue of the periarterial sheaths. During this entire time there is no evidence of any change in the reticular cells of the red pulp, and no mitoses are observed in them. The monocytes present in the spleen develop by individual hypertrophy and transformation of the lymphocytes.

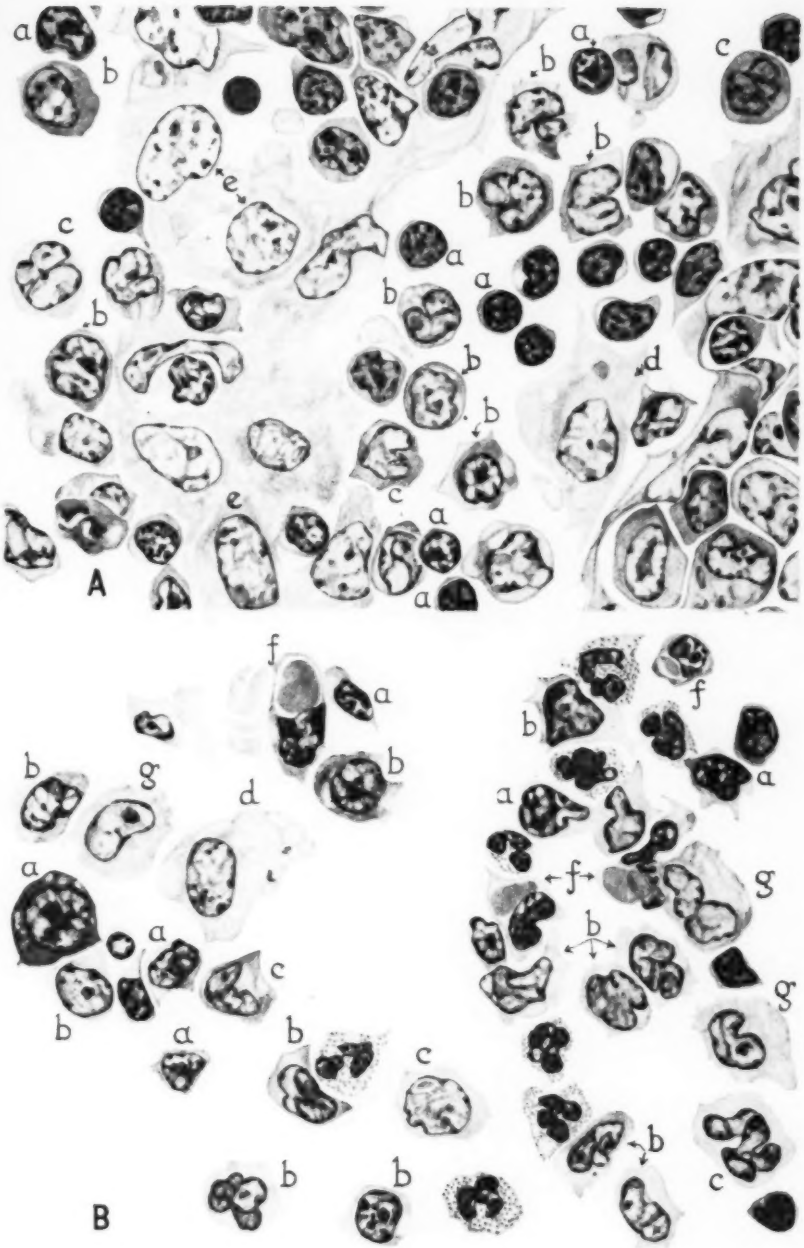
Mesenteric Lymph Node.—In the control node most of the nodules were small and were confined mainly to the outer cortical zone, with a few along the corticomedullary junction. Pale-staining centers were present in the majority of them and consisted mainly of medium lymphocytes with a few mitoses. There were a few which had small centers of reticular cells. The diffuse lymphatic tissue of the cortex was a fairly uniform mass of medium-sized and small lymphocytes and a few scattered large basophil lymphocytes, with here and there an inconspicuous reticular cell. The subcapsular sinus was not distended and contained mainly smaller lymphocytes. The cells in the medullary cords and in the sinuses were small and medium-sized lymphocytes, some macrophages and only an occasional heterophil leukocyte.

At one hour after injection of the infecting dose of Bact. monocytogenes, the even distribution of lymphocytes in the diffuse tissue of the cortex, as observed in the control mesenteric lymph node, was lacking. The lymphocytes at the margins of the nodules in the outer cortex were ameboid. There were irregular dense accumulations of medium-sized lymphocytes in the midcortical and corticomedullary regions. There were more macrophages and red blood cells in the sinuses than in the normal node. The nodules showed no change.

At three hours, in many places in the diffuse cortical tissue the reticular cells were prominent, there being few small and medium-sized lymphocytes in these areas. The nodules in the outer cortical zone for the most part had centers of reticular cells, a few free macrophages and very few lymphocytes; only an occasional nodule in this area showed lymphocytes in mitosis. The subcapsular sinus contained many more cells, which were predominantly medium-sized lymphocytes. The corticomedullary region contained more nodules than previously and these varied considerably in their structure. A few of these nodules were small foci of medium-sized lymphocytes without a sharp outer zone of small lymphocytes. These small foci contained varying numbers of mitotic figures. The larger areas of proliferating medium-sized lymphocytes in this region were circumscribed by densely packed small lymphocytes. But all of the nodules in the corticomedullary region were actively lymphocytopoietic. The free macrophages were very prominent in the sinuses throughout the node but especially in the medulla. They contained much waste pigment and cellular débris.

At six hours, the diffuse lymphatic tissue of the cortex was again densely packed with small and medium-sized lymphocytes and some large lymphocytes. The larger nodules in the cortex had large pale-staining centers; in some of these, part of the pale area contained prominent reticular cells, some dead cells and macrophages, while the other portion was filled with closely packed medium-sized and larger lymphocytes, some of which were in mitosis. Others of these pale-staining centers consisted mainly of medium-sized and large lymphocytes, again with many in mitosis, while a few others were reaction centers, i. e., with few lymphocytes, prominent reticular cells, some macrophages and cellular débris, with no central areas of proliferating lymphocytes. There were in addition several very large homogeneous nodular areas made up entirely of medium-sized and large lymphocytes, with many in mitosis. These large lymphocytopoietic nodules were not circumscribed by a zone of small lymphocytes.

Most of the nodules in the corticomedullary region were smaller than those in the rest of the cortex, and nearly all had an outer dark-staining zone of small lymphocytes surrounding a paler-staining central area of medium-sized lymphocytes. In this region, as well as in the diffuse lymphatic tissue of other parts of the cortex described, there were small and large areas of medium-sized lymphocytes with many mitotic figures which were not demarcated from the adjacent



EXPLANATION OF FIGURE 1

A, camera lucida drawing of a medullary sinus of a mesenteric lymph node of a guinea-pig killed twenty-four hours after an injection of Bact. monocytogenes.

B, camera lucida drawing of a venous sinus of the spleen of a guinea-pig killed forty-eight hours after an injection of Bact. monocytogenes.

In *A* and *B*, *a* indicates lymphocytes; *b*, monocytoid lymphocytes; *c*, monocytes; *d*, macrophages; *e*, reticular cells; *f*, Kurloff bodies; *g*, *monocyte→macrophage*. Hematoxylin-eosin-azure II; × 2,240, reduced to 1,490.

diffuse lymphatic tissue by a zone of small lymphocytes. Lacking this outer zone of small lymphocytes, they may be considered as "bare" lymphocytopoietic nodules in the diffuse lymphatic tissue, in contrast to similar foci which appeared in pre-existing nodules and thus were covered with a layer of small lymphocytes.

Because of the increased lymphocytopoietic activity at this hour, more lymphocytes were produced than left the organ. The sinuses contained large numbers of medium-sized and small lymphocytes and a few free macrophages filled with cellular débris.

At nine hours, the number of larger lymphocytes in the diffuse lymphatic tissue of the cortex was increased. The numerous nodules in the outer cortical zone were large, and most of them consisted chiefly of medium-sized and very large lymphocytes; mitoses in the lymphocytes were numerous. Most of these nodules had no outer limiting zone of small lymphocytes; a few of the larger ones had outer dark-staining zones of small lymphocytes and pale-staining central areas consisting of reticular cells, macrophages, a few lymphocytes and cellular débris; these were "reaction centers." In a few of them there were eccentric areas of medium-sized lymphocytes, some of which were in mitosis. A further variation in nodular structure was the appearance of an eccentric pale-staining zone of proliferating medium-sized lymphocytes in a nodule consisting elsewhere only of small lymphocytes. Again, as at six hours, rounded dense areas of proliferating medium-sized lymphocytes, which bore no relation to a preexisting nodule, could be found in both cortex and medulla.

The medullary cords and sinuses were so crowded with medium-sized and small lymphocytes that the normal architecture was obscured. Many of the medium lymphocytes in the medullary sinuses were in mitosis. There were many free macrophages throughout the node, and eosinophil leukocytes were numerous. Again, as in the one-hour node, there were irregular dense accumulations of ameboid medium-sized and small lymphocytes infiltrating the midcortical area; they showed no evidence of multiplication in this place.

At twelve hours, reaction centers of the type described were beginning to appear in the larger nodules in the cortex. The number of mitoses in the nodules was less than at the previous stage, and reticular cells were more prominent in all of them. Only a few smaller nodules had central areas of medium-sized and larger lymphocytes. In the diffuse tissue of the cortex the cellular stroma was quite prominent; the predominant cells were the large lymphocytes, and these were also numerous in the medulla. The subcapsular sinus had many medium-sized and small lymphocytes as well as monocytoid lymphocytes with small lymphocyte nuclei and a considerably increased amount of cytoplasm. Mitoses were frequent in both medium-sized and large lymphocytes in the medullary sinuses; here plasma cells were also numerous.

At eighteen hours, the lymph node was smaller than at earlier stages, and its cortex was narrow. The nodules in the cortex were numerous but small, and most of them consisted of large outer zones of small lymphocytes with lighter-staining central areas. In all of these pale centers the medium-sized and large lymphocytes were the predominant cells, although the ratio between the two sizes differed in each nodule. In some nodules there were few "tingible bodies" and an occasional macrophage. A few of the larger nodules in the cortex were compact masses of medium-sized and large lymphocytes not demarcated by layers of small lymphocytes from the diffuse lymphatic tissue.

The diffuse lymphatic tissue of the cortex was fairly well filled with small and medium-sized lymphocytes, but in the medulla there were few lymphocytes of any size in either the cords or the sinuses (especially the latter). The reticular

cells of the medulla were prominent. There were several irregular dense accumulations of smaller lymphocytes in the corticomedullary region, similar to those described nine hours after the injection. Plasma cells were numerous in the medullary cords, and there were a few free macrophages in the sinuses.

At twenty-four hours, in sharp contrast to the previous stage, the mesenteric lymph node was large and had many nodules in both cortex and medulla. These varied in size from small isolated areas of proliferating medium-sized lymphocytes in the medulla to the very large nodules in the cortex. Many of the large nodules, with pale-staining central areas consisting of medium-sized and large lymphocytes with many mitotic figures, had dense peripheral zones of smaller lymphocytes, while other large areas of proliferating lymphocytes lacked this dark-staining outer zone. The number of dividing lymphocytes within the nodules was far greater than the number in the diffuse lymphatic tissue outside of the nodules in both cortex and medulla. The medullary cords and sinuses were again crowded with lymphocytes of all sizes and free macrophages. In the sinuses a few large free macrophages with débris-laden cytoplasm were in mitosis. Here there were also a number of monocytes and atypical cells which could not be classified as either monocytes or lymphocytes. Most of these had more cytoplasm than lymphocytes, although they still had lymphocytic nuclei, while in others with less cytoplasm, the nuclear pattern was changed in that the chromatin particles were in smaller clumps along the thinner nuclear membrane. These were classified as monocytoid lymphocytes (fig. 1 *A*). These cells had not been present in the lymphatic nodules but had been increasing in number in the subcapsular sinus, which contained some recognizable monocytes as well as many of these monocytoid lymphocytes.

At thirty-six hours, the corticomedullary junction contained several large, irregular, dense accumulations of small and medium-sized lymphocytes. The smaller lymphoctes were the predominant cells in the diffuse cortical tissue. Pale-staining areas of reticular cells were present in some of the lymphatic nodules, but for the most part the nodules were smaller and lymphocytopoietic. Small areas of proliferating medium-sized lymphocytes with or without peripheral zones of small lymphocytes were scattered through the medulla. The subcapsular sinus contained many lymphocytes, mainly of the smaller type, a few monocytes and many monocytoid lymphocytes. The medullary cords were crowded with lymphocytes, and the sinuses had many monocytoid lymphocytes. Lymphocytes of all sizes showed these monocytoid characteristics. The sinuses were full of red blood cells, but there did not seem to be any appreciable erythrophagocytosis by macrophages. Most of the macrophages were free from pigment and cellular débris.

At forty-three hours, in a few areas of the cortex the reticular cells were prominent. There were few small lymphocytes here, but there were many macrophages and large lymphocytes in this region. In some cortical areas there were many large lymphocytes and some intermediate forms between these and reticular cells, while in other portions of the cortex were dense masses of small and medium-sized lymphocytes. The nodules of the cortex were, for the most part, large and had lymphocytopoietic centers; there were a few smaller foci of proliferating lymphocytes not demarcated from the surrounding diffuse lymphatic tissue.

The medulla had several very large, more or less circumscribed nodules, made up mainly of medium-sized lymphocytes with many mitoses. The medullary cords were filled with larger lymphocytes, while those in the sinuses were smaller. Here also were many macrophages, some monocytes and a few monocytoid lymphocytes.

The subcapsular sinus had many monocytes, and there were whole groups of small lymphoid cells with increased amounts of cytoplasm whose nuclei showed rather marked indentations and a dispersion of the larger chromatin particles. In others of these the nucleoli were less prominent. Accompanying these groups of monocytoid lymphocytes were numerous typical small lymphocytes. These monocytoid groups contained all transitions from typical small lymphocytes to obvious monocytes.

At forty-eight hours, the stroma throughout the greater part of the cortex was very prominent, and the number of lymphocytes here was markedly decreased. The margins of most of the nodules in the cortex were sharply demarcated by a reticular stroma. Some of the nodules consisted mainly of medium-sized lymphocytes, with some in mitosis, while others had proliferating medium-sized lymphocytes together with macrophages containing pigment and red blood cells. There was no evidence of reticular cells of the cortex outside the nodules transforming into the many large lymphocytes present throughout the node. In the medulla there were numerous foci of proliferating medium-sized and large lymphocytes. Some of these areas had outer zones of smaller lymphocytes, while others were either cricumscribed by the reticulum of the cords or were not demarcated from the surrounding tissue. The medullary sinuses and the subcapsular sinus contained many erythrocytes, while the number of monocytes and monocytoid lymphocytes, especially in the subcapsular sinus, was less than at forty-three hours.

At three days, the lymph node was extremely small, and the cortex contained only a few nodules. These had outer zones of small lymphocytes with central areas of medium-sized and small lymphocytes. There were no monocytes here and only a few in the subcapsular sinus. A few of the medium-sized lymphocytes in the nodules contained Kurloff bodies. The diffuse lymphatic tissue of the cortex was very loose. The free cells were smaller lymphocytes and many monocytoid lymphocytes. The cortical sinuses contained mainly smaller lymphocytes, while those of the medulla had, in addition, large numbers of macrophages.

At three and a half days, the reticular stroma was greatly thickened. This was due to a decrease in the size of the node. It was obvious that this was not the result of hyperplasia of the reticular cells but of a prominence of the reticular cells due to the depletion of free cells in the meshes. There were only a few very large nodules in the outer cortical zone, with eccentric areas of lymphocytic proliferation. The majority of the few nodules present in the node at this time were in the medulla or the corticomedullary region. There were many very large lymphocytes in the diffuse cortical tissue. Some of the reticular cells here had large acidophil nucleoli, while others had basophil cytoplasm. In these areas there were all stages in the transformation of the reticular cells of the diffuse lymphatic tissue into large lymphocytes. There was an occasional reticular cell in mitosis, but the greater majority of the dividing cells were lymphocytes. Nearly all of these mitoses were extranodular.

It is significant that at this time, during the height of the monocytosis in the peripheral blood, many of the reticular cells throughout the diffuse lymphatic tissue were transforming into large and medium-sized lymphocytes. This extensive transformation of reticular cells into lymphocytes occurred only after exhaustive lymphocytopoiesis in the lymphatic nodules. Up to this time the intense lymphocytopoiesis by proliferation of lymphocytes had sufficed to produce enough lymphocytes to meet the

need for cells of this type, but the depletion of lymphocytes from the diffuse and nodular lymphatic tissue was now so pronounced that the reserve was gone, the normal mode of lymphocyte production was insufficent and the reticular cells were activated. That they were transforming into lymphocytes and not into monocytes was shown by the finding of all stages intermediate between reticular cells and lymphocytes. In addition, throughout the cortex all stages of transition forms between these lymphocytes and monocytes were present.

There were irregular dense accumulations of medium-sized and small lymphocytes in the inner cortical zone. The medullary sinuses contained many small lymphocytes and macrophages, but the cords were crowded with lymphocytes. The reticular cells were prominent here, and occasionally one could be found in division. There were several heterophil myelocytes in the medullary sinus. At this time the small lymphocytes and monocytes in the subcapsular sinus were connected by a complete series of transition forms.

At four days, the lymph node was somewhat larger, and the reticular cells of the cortex were much less prominent than at three and a half days because of an increase in the number of lymphocytes. Most of these had developed from the reticular cells, which were described in the preceding stage as developing into lymphocytes. There were few lymphocytopoietic nodules in the cortex. The medulla had a few smaller foci of lymphocyte proliferation, and the lymphocytes at the margins of these were ameboid. The medullary sinuses and cords had many smaller lymphocytes.

At five days, the lymph node was larger than at four days. In a few localities in the cortex the reticular cell stroma was again prominent, but the greater part of the diffuse tissue was crowded with lymphocytes of all sizes; here the reticular cells were inconspicuous. Throughout the cortex and medulla were numerous nodules which varied greatly in structure and in size. A few larger ones consisted entirely of proliferating medium-sized lymphocytes; others had centers of dividing medium-sized and large lymphocytes, while a few contained reaction centers. Throughout the cortex were many small nodular areas of medium-sized lymphocytes, many of which were in mitosis. These appeared to be new lymphocytopoietic nodules forming in the diffuse lymphatic tissue. The sinuses and medullary cords had many macrophages filled with a débris of red and white blood cells. There were some monocytes here and in the subcapsular sinus.

At six days, the sinuses throughout the node, as well as the diffuse lymphatic tissue, were more densely crowded with lymphocytes than at five days, but the number of nodules in the outer cortical area was not as great. In the corticomedullary region and in the medulla the nodules were large and consisted mainly of medium-sized lymphocytes, many of which were in mitosis. In the lymphocytopoietic centers within several nodules in the cortex there were a few early heterophil myelocytes arising from medium-sized lymphocytes.

At seven days, the mesenteric lymph node was larger than at any previous time during the infection or in the controls. The diffuse lymphatic tissue of the cortex was densely packed with medium-sized and small lymphocytes and many larger lymphocytes, and the cortical sinuses were crowded with small lymphocytes. The cortex had become progressively larger since the fourth day. The nodules were numerous throughout the cortex and the medulla. Some of these in the cortex

had several cycles of lymphocytopoiesis, i. e., a central light-staining zone of medium lymphocytes surrounded by smaller ones, and this in turn had one or more peripheral zones of larger lymphocytes.

Some of the nodules had medium-sized lymphocytes, some of these in mitosis, and in addition these areas contained varying numbers of monocytes. The subcapsular sinus also contained monocytes, monocytoid lymphocytes and lymphocytes. Some of the smaller nodules in the medulla had pale central zones of reticular cells and macrophages with darker peripheral zones of small lymphocytes, but the majority of the nodules in the medulla consisted mainly of medium-sized lymphocytes, with many in mitosis. Some of these areas of lymphocyte proliferation had limiting peripheral zones of smaller lymphocytes, while many other areas lacked this zone. The medullary sinuses contained macrophages, many lymphocytes and some monocytes.

Summary.—The mesenteric lymph node of the guinea-pig, like the spleen, responds to the injection of Bact. monocytogenes with a movement of lymphocytes from the diffuse lymphatic tissue. Nine hours after the injection this initial depletion is compensated by extensive new formation of lymphocytes. At the end of the first day and during the entire second day these reserve and newly formed lymphocytes are connected with typical monocytes by an intimate series of transition forms. By the end of the second day the depletion of lymphocytes is so extensive that the new formation of these cells does not keep pace with it, and the cortex becomes thin and the node small. This decrease in size becomes progressively greater during the next twelve hours so that on the third day the lymphatic nodules are exhausted of lymphocytes. But even at this time there is no evidence of any cells other than lymphocytes being the source of the many monocytes present throughout the node.

During the succeeding four and a half days there is progressive recovery from the depletion present three days after the injection. This takes place first by individual transformation of reticular cells into lymphocytes as found in the guinea-pig killed three and a half days after infection. During the fourth day many new lymphatic nodules appear, both in the cortex and in the medulla. By seven days the lymph node is more than twice the size of that in the control animal; the cortex and, to a lesser extent, the medulla are crowded with lymphocytes of all sizes. Lymphocytopoiesis is very extensive in the many nodules throughout the node.

Cervical Lymph Node.—In the first twelve hours after the injection of Bact. monocytogenes the cervical lymph nodes showed little or no change from those of the control guinea-pigs. There was an occasional monocyte in the medullary sinuses and a few heterophil leukocytes; cells of these types were also found in the control animals. During the next twelve hours the number of monocytes in the sinuses increased appreciably; a few of them contained Kurloff bodies.

At thirty-six hours large groups of plasma cells and of myelocytes were present in the inner cortical zone and in the medullary cords; erythrophagocytosis

by the macrophages of the sinuses was marked. Monocytes were present throughout the medullary portions of the node. There were many medium-sized lymphocytes in mitosis in the lymphatic nodules, but the cervical lymph nodes did not show the cyclic changes of the mesenteric group. The depletion of lymphocytes from the cortex of the node was not nearly as marked here as in the mesenteric node.

In the animals killed five days after the injection (at the time when the mesenteric lymph nodes were increased in size) the cortex of the cervical node was also greatly increased. There were many more lymphocytopoietic nodules than previously. The medullary cords were distended, and the sinuses were crowded with cells. Among these, the medium-sized and large basophil lymphocytes were prominent, and both types showed many mitoses. The large free macrophages were filled with engulfed erythrocytes. Heterophil and eosinophil myelocytopoiesis was marked in the diffuse lymphatic tissue and occasionally was found in the nodules. Most of the early myelocytes of these two groups had lymphocytic nuclei.

The cervical lymph node on the sixth day after the injection showed extensive myelocytopoiesis, both from lymphocytes and from the reticular cells; also some megakaryocytes were present in the medulla.

OBSERVATIONS ON THE PERIPHERAL BLOOD OF INFECTED RABBITS

The blood serum of the rabbits, taken before the injection, showed no agglutination in dilutions from 1 : 10 through 1 : 5,120 with either the avirulent stock strain of Bact. monocytogenes or with the strain virulent for rabbits.

The average white blood cell count before the injection was 12,500 cells per cubic millimeter. Fluctuations in the total white cell counts similar to those noted for the guinea-pigs during the first twelve hours also occurred in the rabbits. Leukopenia was present also from eighteen to thirty-six hours after the injection, at which time the white cell count was 6,300. Following this the count increased to 19,000 at forty-eight hours and maintained that level at sixty-six hours.

The heterophil leukocytes and the lymphocytes showed approximately the same variations in number as those found in the guinea-pig.

The average percentage of monocytes in the animals before the injection was 3 per cent. The three and the six hour rabbits had no monocytes in the peripheral blood. From nine to thirty-six hours after the injection the monocytes numbered about 6 per cent of the total number of leukocytes. At forty-eight hours they increased to 26 per cent and at sixty-six hours, at the time the number of lymphocytes was low (15 per cent), they increased to 54 per cent of the total number of leukocytes. The basophil leukocyte count constituted 9 per cent of the total number of leukocytes twenty-four hours after the injection.

The heterophil leukocytes in the peripheral blood of the rabbits killed at forty-eight and sixty-six hours after the injection had many toxic granules; some of the monocytes at these same times contained phagocytosed erythrocytes, and the cytoplasm of others was markedly vacuolated. The red blood cells showed no apparent change during the first twenty-four hours following the injection, but at thirty-six hours poikilocytosis was apparent, and some normoblasts were present. At forty-eight hours there was a marked increase in the number of normoblasts; polychromatophilia was pronounced; there was marked basophilic stippling, and anisocytosis and poikilocytosis were increased in degree as well as in frequency.

GROSS ANATOMIC OBSERVATIONS ON INFECTED RABBITS

During the first twenty-four hours after the injection of Bact. monocytogenes there was a marked increase in the size of the mesenteric lymph node. A decrease in size was apparent at thirty-six hours, and this decrease continued until at sixty-six hours the mesenteric lymph node was so small that an accurate measurement could not be obtained. The brown color which it presented grossly was the normal color of the medulla.

The spleen was also increased in size, but, unlike the mesenteric lymph node, it was even larger during the second day than the first. At sixty-six hours it was blue-red but was not as swollen as the spleen of the forty-eight hour rabbit.

The bone marrow of the femur at thirty-six hours was dark red and quite adherent to the endosteum, while at forty-eight hours it was adhesive along the entire length of the marrow cavity and showed several small gray areas. This adhesiveness was not apparent at sixty-six hours.

MICROSCOPIC OBSERVATIONS ON INFECTED RABBITS

Spleen.—In the spleen of the normal rabbit there was a fairly sharp demarcation between the white pulp and the red. In cross-section the periarterial sheaths were comparatively sharply circumscribed, and the lymphatic nodules within them were for the most part pale-staining, and their centers consisted mainly of reticular cells. The cords and sinuses were distinct, and most of the cells here were small and medium-sized lymphocytes with an occasional monocyte and some heterophil leukocytes.

The spleen of the rabbit studied during the early stages of infection with Bact. monocytogenes up to the time of the appearance of great numbers of monocytes in the peripheral blood showed an initial response to the infection essentially the same as that described for the spleen of the guinea-pig. The rapid early and continued migration of lymphocytes from the white pulp into the red was accompanied by an extensive new production of lymphocytes in the periarterial nodular lymphatic tissue. The latter process did not begin until twelve hours after the injection in the guinea-pig and reached its height in twenty-four hours, compared with eighteen hours in the rabbit. The continued migration of lymphocytes preexisting in the white pulp, as well as of those newly formed, was the source of the many lymphocytes found in the cords and sinuses of the red pulp during the second day. As described for the guinea-pig, at this time there was individual transformation of lymphocytes into monocytes; forms representative of all stages of this transition (monocytoid lymphocytes) were present in the red pulp. There were also many monocytes in the nodules of the white pulp (fig. 2). Up to this time there was no evidence of changes in the reticular cells of the red pulp.

Unlike the guinea-pig, the rabbit at forty-eight hours after the injection showed the lymphatic tissue of the periarterial sheaths more extensive than at thirty-six hours, and many nodules which were lymphocytopoietic. Throughout the diffuse lymphatic tissue of the sheaths were numerous large lymphocytes; many of these were developing from reticular cells. This process also appeared to be taking place in the red pulp but to a far smaller degree. Many of the reticular cells of the red pulp were very prominent, and several were in mitosis. This may have indicated that, though the lymphocytopoiesis of the previous thirty-six hours had been intensive, the lymphocytes preexisting in the lymphatic tissue could not produce sufficient lymphocytes to meet the demand and the reticular

cells of the red pulp were activated. But the greatest amount of lymphocyte production was still confined to the white pulp as evidenced by the greater number of mitoses in the latter.

Sixty-six hours after the injection the nodules, as well as all of the periarterial sheaths in the spleen, appeared depleted of lymphocytes, and their margins consisted mainly of monocytoid lymphocytes and monocytes. In one large nodule the central zone had many macrophages, dead lymphocytes and epithelioid reticular

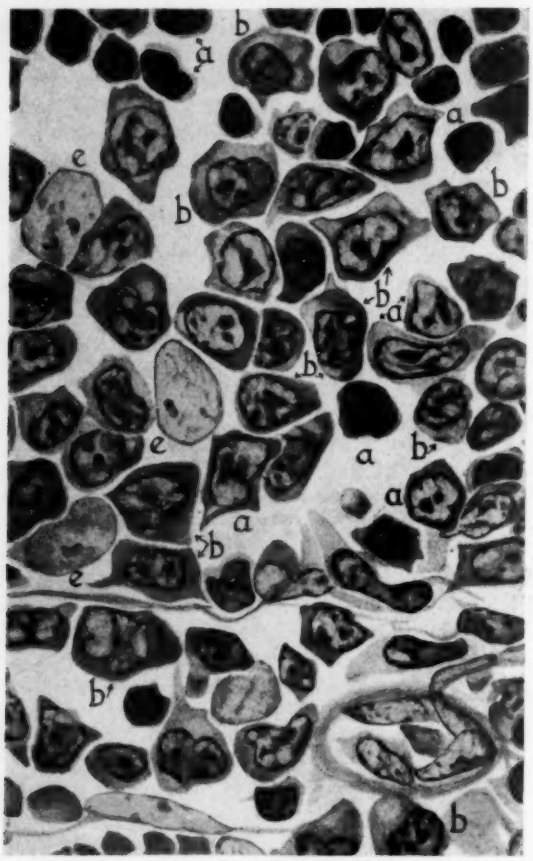


Fig. 2.—Group of monocytoid lymphocytes at the edge of the periarterial lymphatic tissue of the spleen of a rabbit killed forty-eight hours after an injection of Bact. monocytogenes. The small letters and the optical system have the same explanation as in figure 1.

cells, as well as large lymphocytes, with many cells intermediate between these and the reticular cells. In addition, the entire nodule was infiltrated with heterophil leukocytes, many of which were dying.

Throughout the red pulp were numerous islands of heterophil myelocytes. Many of them were free and had nuclei like those of the reticular cells, while some were still attached. In a few areas there were groups of developing

megakaryocytes. The lining cells of the sinuses in the red pulp were actively phagocytic; they contained red blood cells and fragments of dead leukocytes.

More reticular cells of the cords in the red pulp were in mitosis than at forty-eight hours. There were numerous intermediate stages in the formation of large lymphocytes from the reticular cells of the Billroth cords.

It should be mentioned that this is the stage in the infection at which most of the previous workers began to study the organs of their infected rabbits, for at this time the number of monocytes in the peripheral blood is greatly increased. It is obvious that if one started to examine the spleen at this time, when the entire red pulp contains numerous monocytes, the reticular cell proliferation present at this time might give the erroneous conclusion that the monocytes have their origin in the reticular cells (reticuloendothelial). But the series of events leading up to this hour demonstrate, without doubt, that this activation of reticular cells takes place only after exhaustive lymphocytopoietic activity on the part of the lymphocytes in the diffuse and nodular lymphatic tissue of the periarterial sheaths. The monocytes which are present in the spleen in great numbers at this and slightly earlier stages are connected by an intimate series of transition forms with the many lymphocytes filling the red pulp following migration into it from the sheaths. Even at this time the reticular cells of the Billroth cords are developing, not into monocytes, but into large lymphocytes. This is evident from the many transition stages between these two cell types which are present, and in addition throughout the red pulp there are still many large and medium-sized lymphocytes which are becoming monocytoid.

Mesenteric Lymph Node.—In the control the diffuse lymphatic tissue of the cortex was compactly filled with small and medium-sized lymphocytes. Lymphatic nodules were numerous in the outer cortical zone, and most of these consisted of more or less densely packed small lymphocytes (fig. 3*A*). Only an occasional nodule here had a pale-staining central area of either reticular cells or medium-sized lymphocytes. In the corticomedullary region and in parts of the medulla there were a few of these large nodules, but most of the nodules here were smaller and contained more proliferating medium-sized lymphocytes in their paler-staining centers. The medullary cords and sinuses had only a moderate number of smaller lymphocytes and some macrophages. Eosinophil leukocytes were present here and there throughout the node.

The initial reaction of the mesenteric lymph node of the rabbit to Bact. monocytogenes was essentially the same cytologically as in the guinea-pig; there were mobilization of existing lymphocytes and migration of these cells from the cortex, crowding the sinuses and medullary cords. This migration was accompanied by intensive new formation of lymphocytes, as shown by the great increase in numbers of lymphatic nodules in which numerous mitoses of lymphocytes were found (fig. 3*B*). There were few or no mitoses in the diffuse lymphatic tissues. During the first twenty-four hours more lymphocytes were produced than left the node, and it accordingly increased in size. During the second day the node decreased in size, most of the nodules in the cortex were depleted of lymphocytes, and many reaction centers appeared (fig. 3*C*). This lymphocytopoiesis in the

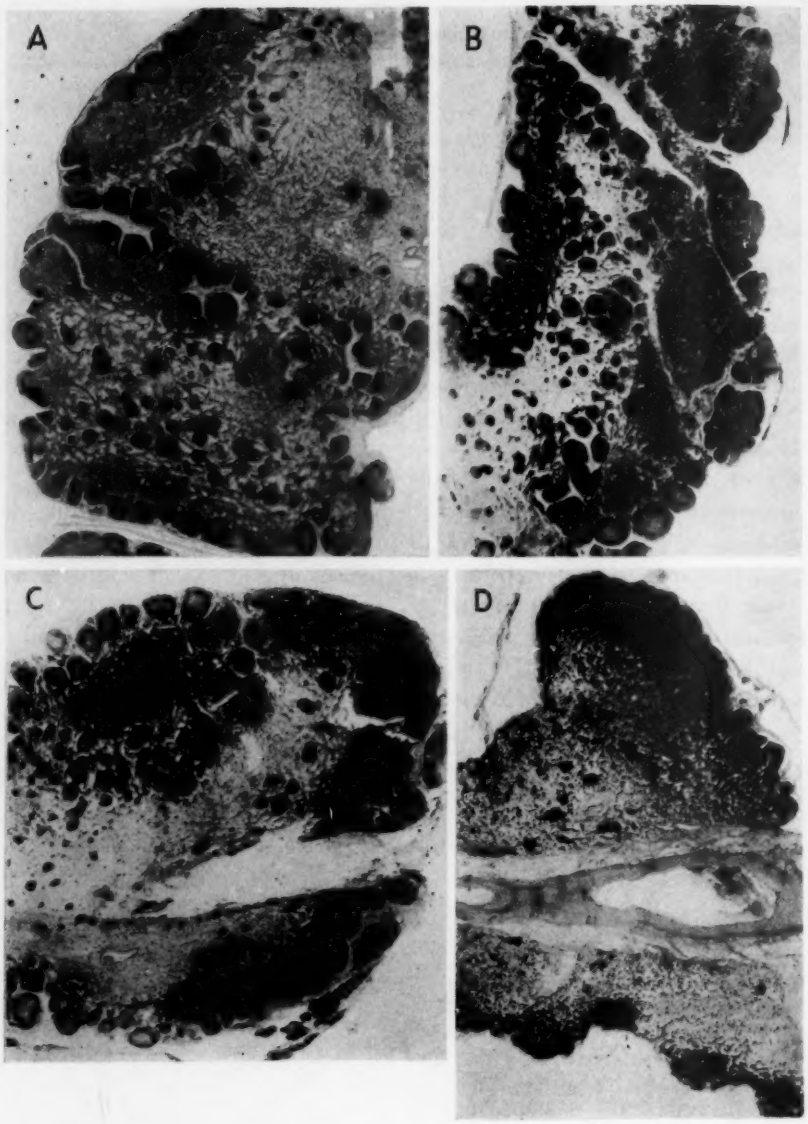


Fig. 3.—Photomicrographs of mesenteric lymph nodes of rabbits after an injection of Bact. monocytogenes. *A*, control, an uninfected rabbit; *B*, infected rabbit killed eighteen hours after the injection; *C*, infected rabbit killed thirty-six hours after the injection; *D*, infected rabbit killed sixty-six hours after the injection. Note the disappearance of the diffuse lymphatic tissue of the cortex and of the nodules in *D*. Here loose lymphatic tissue predominates and often reaches the subcapsular sinus. Hematoxylin-eosin-azure II; × 12, reduced to × 8.

nodules did not produce sufficient numbers of lymphocytes; the cortex became so depleted of lymphocytes that by the third day little diffuse lymphatic tissue remained and most of the node was composed of loose lymphatic tissue resembling that normally found in the medulla (fig. 3 *D*).

At the end of the first day and during the second day before the height of monocytosis in the peripheral blood, there were many transition stages between lymphocytes and monocytes throughout the node. During these early stages of the infection, as well as on the third day, there was no evidence that any cells other than lymphocytes were the source of the many monocytes found in the lymph node thirty-six and forty-eight hours after the injection of Bact. monocytogenes.

Cervical Lymph Nodes.—The cervical lymph nodes of the rabbit did not show the cyclic changes described for the mesenteric group. The depletion of lymphocytes from these nodes was not pronounced nor was there extensive new formation of lymphocytes as in the mesenteric group. But an increase in the number of monocytes began at eighteen hours after the injection of Bact. monocytogenes and became progressively more extensive through the second day. During this time all transitions between lymphocytes and monocytes were present throughout the nodes.

COMMENT

The diverging and at times diametrically opposed results in previously reported studies to determine the source of the monocytosis characteristically caused by Bact. monocytogenes are due in great part to the failure to make cytologic studies of the organs of the infected rabbits at close intervals shortly after the injection of the bacteria. In my experiments it has been clearly demonstrated that during the forty-eight hours previous to the height of the monocytosis of the blood evoked by Bact. monocytogenes the organism produces great numbers of monocytes through individual transformation of the lymphocytes present in the cortex of the mesenteric lymph node and in the periarterial lymphatic tissue of the spleen.

The first stage in the monocytopoiesis is a widespread migration of lymphocytes from these areas of lymphatic tissue during the first nine hours after the injection, which is quickly compensated by greatly increased new formation of lymphocytes. In the guinea-pig this is considerably more marked and appears earlier in the mesenteric lymph node than in the spleen, while in the rabbit there is little difference in time between the reactions in the two organs. This great new formation of lymphocytes, especially in the spleen, is the source of the vast numbers of large lymphocytes crowding the cords and sinuses of this organ about twenty-four hours after the injection.

As early as forty hours before the height of the monocytosis in the peripheral blood is reached, there are many groups of lymphoid cells, particularly in the spleen but also in the mesenteric lymph node, which have more cytoplasm than the smaller lymphocytes. As contrasted with typical lymphocytes, these cells show progressive increases in the inden-

tation of the nucleus, the chromatin particles are more finely divided, and the nucleoli are smaller and more numerous. These changes in the nucleus as well as in the nucleus-cytoplasmic relationship are found in such varying degrees in so many lymphocytes that they constitute an intimate series of transitions from lymphocytes to monocytes. The presence of these transition stages is of particular significance when they are considered in relationship to the time of their appearance. In the early stages of the infection, lymphocytes predominate; between eighteen and twenty-four hours after the injection the first stages of the transition forms appear. At this time only a few monocytes are present. As the process extends, more monocytoid lymphocytes and typical monocytes are present and the number of lymphocytes decreases sharply.

These "monocytoid lymphocytes" are identical with those described by Bloom [2] in the sinuses of the spleen and liver of the rabbit during the height of infection with Bact. monocytogenes. The lymphocyte nature of these morphologic lymphocytes cannot be denied simply because they give rise to monocytes. To do this one would have to postulate that either something had happened to the free cells of the lymphatic tissue and that "monoblasts" had suddenly replaced all of the lymphocytes, or that the "monoblasts" are morphologically identical with the adjacent lymphocytes of the lymphatic tissue. Throughout the initial stages of this infection there are many mitoses in lymphocytes but none in reticular cells. During this time there is no evidence of the rounding up of these fixed cells in either the spleen or the lymph node.

Just previous to the peak of the monocytosis in the peripheral blood, many of the lymphatic nodules in the spleen and in the mesenteric lymph node of both the rabbit and the guinea-pig contain monocytes and many monocytoid lymphocytes as well as typical lymphocytes. In some of these nodules the monocytoid lymphocytes and monocytes are confined to the peripheral part of the nodule and the central part contains mainly proliferating medium-sized lymphocytes; in others they are found throughout the nodule.

At the end of the second day and during the third day after the injection of Bact. monocytogenes, in both the rabbit and the guinea-pig, when the monocytosis in the peripheral blood is well developed and near its height, the first evidences of the transformation of reticular cells into cells of any other type are found in the diffuse lymphatic tissue of the cortex of the mesenteric lymph node and of the periarterial sheaths of the spleen. This occurs after the lymphatic tissue is depleted of lymphocytes in spite of the extensive lymphocytopoiesis of the first forty-three hours after the injection of the bacterium. The reticular cells are prominent now because of the depletion of lymphocytes which have left the tissue and become monocytes. That the reticular cells in these areas are transforming into large lymphocytes and not into monocytes is

clearly shown by the presence of numerous intermediate forms between reticular cells and large lymphocytes; in addition, there are all transitions between these lymphocytes and monocytes in the lymphatic tissue. If the early stages leading up to this one had not been studied, these findings at forty-eight hours would give the impression of a reticular cell hyperplasia. Although the lymphocytopoiesis in the lymphatic nodules of both these organs has been intense, the lymphocytes do not produce sufficient new lymphocytes by mitosis. As a result, the reticular cells are now taking an active part in the production of new lymphocytes in the diffuse lymphatic tissue.

In addition, in the rabbit, beginning at forty-eight hours and proceeding more extensively sixty-six hours after the injection, there are numbers of mitoses in the reticular cells of the red pulp of the spleen as well as all stages of transition between these reticular cells and large lymphocytes. Here again, as in the white pulp, if the study of the spleen had been started at this time, the obvious conclusion might be that the reticular cells were the main source of the monocytes, whereas the findings in the spleen leading up to this stage show that the activation of reticular cells in the red pulp takes place only after intense but insufficient lymphocytopoietic activity on the part of the lymphocytes of the white pulp. Even at this time, which is at the height of the monocytosis in the peripheral blood, the reticular cells of the red pulp are also producing lymphocytes and many of the large and medium-sized lymphocytes in the red pulp are becoming monocytoid. That the reticular cells are not transforming into monocytes or monocytoid cells directly is shown by the finding of a great number of cells intermediate between reticular cells and large and medium-sized lymphocytes, as well as by the fact that the monocytoid cells have predominantly lymphocytic nuclei. They do not have the vesicular nucleus of the reticular cell. On a theoretical basis one could postulate the origin of the monocyte from the fixed reticular cell of either phagocytic or undifferentiated nature. But in my material evidence for this transformation is completely lacking in all of the animals examined. Nor do I find any evidence to support the claims that the hemohistioblast of Ferrata or a free reticular cell is the stem cell of the monocyte (Rinehart;[9] Rezzesi[5]). There is no evidence of a transformation of the reticulo-endothelial cells of the spleen, liver and lymph node into these free cells nor of the transformation of the fixed cells directly into "monoblasts" other than lymphocytes.

One of the few points on which most of the previous investigators of the origin of the monocyte agree is the conclusion that the mesenteric lymph node is not involved in the monocytic reaction in either the normal rabbit or that infected with Bact. monocytogenes. Cunningham, Sabin

9. Rinehart, J. F.: Arch. Path. **13**:889, 1932.

and Doan [10] were unable to find any monocytes in the supravital smears of the mesenteric lymph node of the rabbit. Forkner,[11] using his supravital technic, examined many of the various lymph nodes of rabbits, guinea-pigs and rats and concluded that normally monocytes develop constantly in large numbers in all of the lymph nodes of the body except the large mesenteric group. He claims that his monoblast is easily demonstrated in the paraffin section of the supravitally stained peripheral lymph node "by the fact that it is in close association with premonocytes and monocytes in clumps of these cells." He gives no criteria for separating this "monoblast" from the lymphocytes in the lymph node, although he denies that the lymphocytes give rise to the monocytes.

The previous investigators who used Bact. monocytogenes usually described the mesenteric lymph node after the infection had reached its height, in some cases as long as a month afterward. Bloom [2] reported the formation of monocytes in the mesenteric lymph node only in rabbits which had been splenectomized and subjected to injection of india ink into the portal vein and then injection of Bact. monocytogenes. In his normal rabbits after the injection of this bacterium he found that there were practically no monocytes or monocytoid cells in any parts of the mesenteric lymph nodes of rabbits receiving this bacterium alone or in conjunction with lithium carmine. It will be noted that all of his observations on the mesenteric lymph node were made either at the height of the monocytosis in the peripheral blood or subsequent to it, while my experiments have shown that the monocytogenic reaction in the mesenteric lymph node is at its peak prior to that of the monocytosis in the peripheral blood.

Witts and Webb,[12] using supravital preparations of material obtained by puncture of mesenteric lymph nodes, stated that the glands are absolutely destitute of monocytes and monoblasts; they examined the glands ten, twenty-four, forty-eight and seventy-two hours after the infection as well as later. Rezzesi,[5] using the same technic, commented on the fact that the mesenteric lymph nodes are not involved in the general monocytosis. Nyfeldt,[4] in his studies with a strain of Bact. monocytogenes isolated from a human being, agreed in substance with Bloom but described a great proliferation of large lymphoid cells in the mesenteric lymph node. However, he also concluded that this did not partake in the general monocytogenic response.

On the other hand, Levi and Penati [7b, c] studied the organs of rabbits infected with Bact. monocytogenes and described a great increase

10. Cunningham, R. S.; Sabin, F. R., and Doan, C. A.: Contrib. Embryol. **16**:227, 1925.

11. Forkner, C. E.: J. Exper. Med. **52**:385, 1930.

12. Witts, L. J., and Webb, R. A.: J. Path. & Bact. **30**:687, 1927.

in "lymphocytes and hemocytoblasts" in the mesenteric lymph nodes, as well as in the peripheral groups, prior to the peak of the monocytosis. But they concluded that the monocytes arise from the hemocytoblasts as a cell line apart from the lymphocytes, which are developing concomitantly. They base their conclusion that the monocytes are distinct from the lymphocytes on their study of dry smear preparations, stating that this differentiation is not possible in a study of sections or wet fixed smears. They do not, however, give the basis on which they have been able to discriminate between lymphocytes, hemocytoblasts and monoblasts in the dry smear preparations.

Throughout the course of my experiments the most striking feature in the early stages of the infection was the progressive increase, followed by a marked decrease, in the gross size of the mesenteric lymph node, together with the extensive cortical and medullary changes which I have described. This process is present not only in the rabbit, the animal used by the other investigators, but also, very prominently, in the guinea-pig. In rabbits and guinea-pigs killed at close intervals from the time of the injection to the peak of the monocytosis, the monocytes have been shown to develop by hypertrophy and transformation of lymphocytes. Complete series of cells intermediate between lymphocytes and monocytes are present as early as eighteen hours after the injection of Bact. monocytogenes and can be followed in sections as well as in dry and wet fixed smears of the mesenteric lymph nodes. Moreover, in the later stages of the infection in the guinea-pig the mesenteric lymph node, after the phase of extreme depletion, increases to a size far exceeding that of the control.

In the early stages of the infection in both the rabbit and the guinea-pig, the white pulp of the spleen and the cortex of the mesenteric lymph node show strikingly similar reactions. The participation of the spleen in the general monocytosis evoked by the bacterium is admitted by all, but the interpretations of this reaction are almost as numerous as there have been investigators. I believe the explanation of these divergences of opinion of the previous investigators of this subject is that all of them failed to study the tissues in the early stages of the infection.

In the usual defense reactions in the body, fixed macrophages become free and additional macrophages develop from lymphocytes. In focalized areas in heavy infection with Bact. monocytogenes, Bloom [13] described transformation of fixed macrophages into free phagocytes, but he saw no transformation of these macrophages or of the fixed cells into monocytes. In systemic infection with this bacterium, proliferation of the elements of the reticulo-endothelial system cannot be the source of

13. Bloom, W.: Arch. Path. **6**:995, 1928.

the monocytes that crowd the sinuses of the spleen and the lymph nodes. No evidence of the multiplication of reticulo-endothelial cells could be found in any of the early stages leading up the height of the monocytosis in the peripheral blood.

The diversified opinions as to the origin and nature of the monocyte have been reviewed by Maximow,[14] Bloom[15] and Levi and Penati.[7] A survey of the literature shows that the monocytes develop from lymphocytes or at least from cells that cannot be distinguished from lymphocytes by present methods. No one has given morphologic criteria by which all monoblasts can be distinguished from all lymphocytes (and myeloblasts). The separation of the cells on the basis of "their progeny" or of the "company which they keep" cannot be considered morphologic. The concept of a specific monoblast, i. e., a free cell morphologically different from a hemocytoblast, whether that be a myeloblast or a lymphocyte, has not been proved.

The conclusion presented here that the monocytes which are present in great numbers in infection with Bact. monocytogenes arise from lymphocytes is based on three main points: 1. The initial response to the injection of Bact. monocytogenes in both the rabbit and the guinea-pig is a mobilization of the lymphocytes present in the lymphatic tissue, followed by an intensive new formation of lymphocytes. 2. During the height of this initial hyperplasia, which precedes the peak of the monocytosis in the peripheral blood, all stages of transition between lymphocytes and monocytes are found throughout the diffuse and nodular lymphatic tissue of both the mesenteric lymph node and the spleen. 3. There is complete absence of any evidence of cells other than lymphocytes transforming into monocytes.

The sequence of events in the early stages in both the guinea-pig and the rabbit offers further proof of the correctness of the conclusion reached by Bloom[2] that "monoblasts other than lymphocytes do not exist."

CONCLUSIONS

The monocytosis evoked by infection with Bact. monocytogenes is essentially the same in rabbits and in guinea-pigs.

The initial stage in the production of the monocytosis is a mobilization of preexisting lymphocytes, followed by an extensive new formation of lymphocytes in the lymphatic nodules of the mesenteric lymph

14. Maximow, A.: Bindegewebe und blutbildende Gewebe, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, pt. 1, p. 232.

15. Bloom, W.: Folia haemat. **37**:63, 1928; Lymphocytes and Monocytes, in Downey, H.: Handbook of Hematology, Paul B. Hoeber, Inc., New York, 1937.

node and of the periarterial lymphatic tissue of the spleen. The peripheral nodes play a much less important part in the process.

This marked production of lymphocytes occurs in the early stages of the infection, before the height of the monocytosis in the peripheral blood.

The failure of previous investigators to study these early stages explains some of the differences of opinion among them.

The free stem cell from which the monocyte develops in the diffuse lymphatic tissue and lymphatic nodules of the mesenteric lymph node and in the spleen is morphologically identical with the lymphocytes in these tissues.

Monoblasts other than these lymphocytes have not been found.

EVIDENCES OF SYPHILIS IN MOUND BUILDERS' BONES

A GROSS PATHOLOGIC STUDY

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In this era of exact sciences one often finds that one is not satisfied with mere possibilities and suppositions. The purpose of this paper is to offer evidence which tends to remove existing doubt concerning the presence of syphilis in the prehistoric Mound Builder Indians.

The origin of syphilis is probably one of the oldest of disputed questions. There are two major considerations which make unqualified statements difficult: One is the difficulty of establishing the antiquity of the bones in question and definitely dating them before the Columbian period; the other is the difficulty of stating with certainty that the lesion is due to syphilis rather than to pyogenic osteomyelitis with periostitis, leprosy, yaws, actinomycosis, osteitis deformans or even the postmortem changes caused by beetles and weather. It is believed that the first of these points—the antiquity of the Mound Builders' bones—can be definitely established.

Various races of mankind have appeared on earth, remained for a brief span of time, and then met with destruction and extinction. Although valuable information has been gleaned from the fragmentary evidences that have been left by these extinct races, much still has to be left to the imagination.

The abode of the Mound Builders with their remains is all that is left of this early civilization. It is a beautiful frontispiece to a book the pages of which have been blotted out by the ages. Whichever of the many fantastic theories as to the origin of these people is accepted —e. g., the Phenician migration from the east by Bering Straits, a separate origin, inhabitants of Atlantis or the Ten Tribes of Israel—it is admitted, at least, that here once lived and died a great community. From their artefacts of pottery, stone, bone and shell, inferences as to their modes of living can be made. From their bones only a small amount of data concerning their diseases can be determined. This is not

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surprising when one recalls the high death rates from many diseases which cause no changes in the bones. Moreover, the years have made interpretation of all bone changes more difficult.

In arid countries there remains a more nearly complete history of the people. There many artefacts of cloth, skin and wood remain. Many races, such as the ancient inhabitants of Peru and of Egypt, used efficient methods for preserving the dead.

The Mound Builders buried their dead in a horizontal position on the back, with a water bottle and food bowl at the head to furnish nourishment on the trip to the happy hunting ground. In some cases skeletons were found with many bowls, beads and objects of adornment. The burials of the Mound Builders are to be contrasted with those of the Choctaws, who placed their dead in huge burial urns. This permits ready differentiation of the Mound Builders' burials from those of the Choctaws, who inhabited the mounds in later years.

PATHOLOGIC OBSERVATIONS

The mounds from which the bones described in this paper were excavated are located at Moundsville, Ala., which lies about 79 miles (112 kilometers) south of Birmingham. The excavations were carried out under the direction of Dr. Walter B. Jones, state geologist of Alabama, Mr. David L. DeJarnette, curator of the Alabama State Museum and one of us (W. L. H.). Dr. Jones and Mr. DeJarnette, though not primarily interested in osteology, were well qualified to interpret the findings from an archeological standpoint. Each grave was carefully examined and all objects removed and numbered; frequently photographs were taken in situ. The mounds made an advantageous abode for tribes which followed the Mound Builders, and naturally had been used not only for inhabitation but for interment. During the excavations every effort was made to rule out such later burials. The artefacts and bones from the later graves were filed under a separate system. It is quite possible, however, that some graves of the upper levels were misinterpreted (fig. 1). In the case of some of the bones, however, there is one fact which removes all doubt as to the time of interment. Excavations made on top of the mound and extending down to the base or level of the surrounding soil revealed that on several occasions the height of the mounds had been raised. Covering the mound would be layers of broken pottery, mussel shells, fragments of animal bones, charcoal and other material which had collected on the mound during habitation. When from 4 to 10 feet (122 to 305 cm.) of dirt had been removed, it was found that there was another layer of the aforementioned materials covering the entire mound, showing thus the evidence of a second inhabitation. Over many graves no

evidence of disturbed soil was found; below the second or the first level, however, there would be evidences of a burial, consisting of broken shells and charcoal. These were so characteristic of the graves that even the colored men employed in the digging recognized them and often

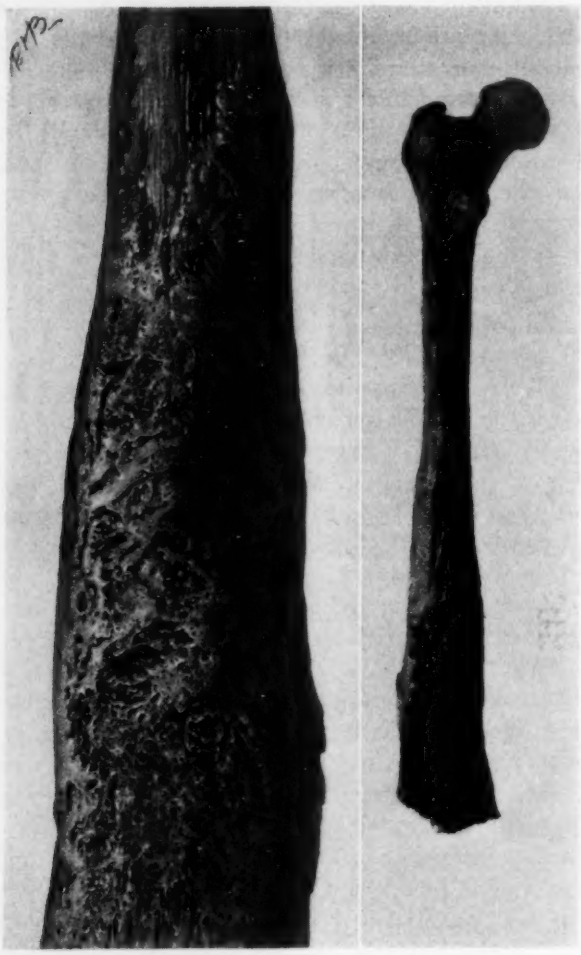

Fig. 1.—Drawing and photograph of specimen 1. Note the evidence of proliferation of the periosteum and thickening of the cortex, with many irregular eroded areas, characteristic of syphilis.

stopped working in test pits in which they were not found. Thus as the deeper levels were reached, the remains of older generations were encountered. None of the later burials, i. e., of Choctaws and others, penetrated below the upper level.

The exact date of the inhabitation of the mounds is questionable, but an approximation can be made from available data. In recorded history there is little to establish the date. When DeSoto made his trip across what is now Alabama in 1530, some of his men described

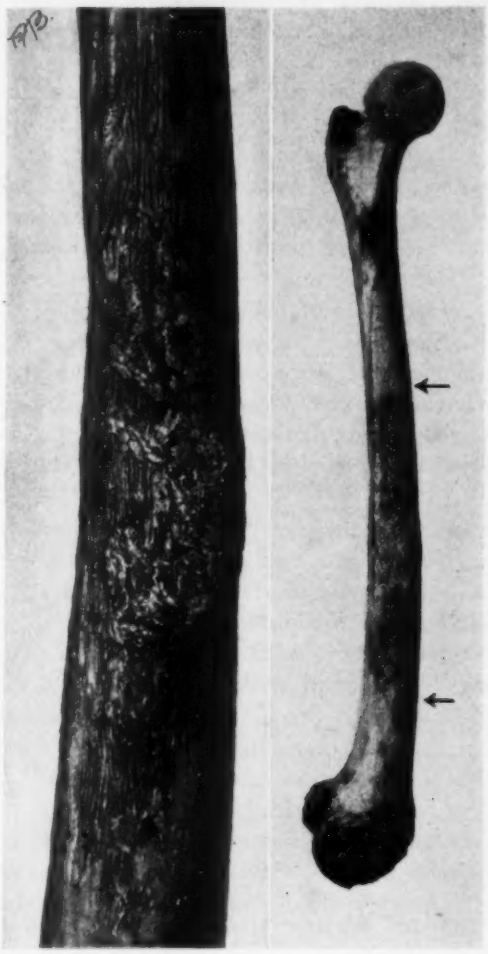

Fig. 2.—Drawing and photograph of specimen 2. Note the evidence of periosteal proliferation localized to one area (indicated by arrows) and very suggestive of syphilis.

briefly articles which they had obtained from the Indians. One states that according to a Choctaw Indian legend, "these mounds were already built and unoccupied when our forefathers came here, many generations ago."

There is much evidence to support the belief that the Choctaw Indians inhabited the region of the mounds for a long period. This alone places the date of the discovery of the mounds by the Choctaws at about 1,000 A. D. The size of the mounds indicates that it would of necessity have required several generations to build them. There are two large mounds about 50 feet (15 meters) in height (fig. 2), surrounded by twenty other still recognizable mounds. The number of cubic yards of dirt involved has been estimated by measuring their height and circumference. The amount one person could carry on his back in a container or drag in skins, the number of trips he could make daily from the ravine from which the soil was obtained, and the number of working days during the year have been estimated. Possibly the assumptions are faulty, as the method of moving the dirt is not known with certainty. From these calculations it has been estimated, however, that it would take 10,000 people one thousand years to build these mounds. The fact that several levels of habitation were found shows that there were a number of successive periods of construction.

From archeological literature one is able to find but few records of mounds which have been erected in Europe or Asia after 1,000 A. D. It is not definitely known, however, whether the age of the Mound Builders in America is contemporaneous with that in the eastern hemisphere.

No designs found on the pottery show any evidence of contact with the white man. No artefacts used by the white man were found in the original burials, but in some of the subsequent interments glass beads and similar objects were found.

The swastika, which is one of the most ancient of designs, is common on the pottery of the Mound Builders. The tribes which built these mounds are unmistakably pre-Columbian. Whatever diseases their bones may show certainly originated with them or were handed down from generation to generation, and were not new scourges spread following the invasion of America by the white man.

The establishment of the diagnosis of syphilis is as nearly certain as the circumstances permit. Several specimens show changes which we believe conform very closely to the classic description of lesions produced by syphilis.

The long bones offer strong evidence of syphilitic changes, but it is realized that they may show pseudosyphilitic changes due to pyogenic osteomyelitis with periostitis, osteitis deformans, yaws and similar diseases.

On the other hand, the skulls offer indisputable evidence. It is interesting, however, that despite the examination of many thousands of well preserved teeth none were found which could be called Hutchinson's teeth.

A brief description of some of the most characteristic specimens follows, including those thought to be affected by syphilis and those showing nonsyphilitic lesions. From the description it will appear that syphilitic and nonsyphilitic lesions can be differentiated with some degree of accuracy. The specimens are well preserved, and their condition can be as accurately diagnosed as any modern bone specimen not accompanied by histologic study or clinical information.

1. *Left Femur* (fig. 1).—This bone weighs 261 Gm. and is 40 cm. long. The angle between the neck and the shaft is approximately normal; the head and neck are somewhat smaller than normal for the size of the shaft. The lower portion of this femur, including the condyles, has been broken off. Above this point, extending up to about the middle of the bone, and surrounding its surface on all aspects, is an extensive area of proliferative periostitis and new bone. There are numerous indentations and irregularities throughout this area; these are most marked on its posterior aspect. Near the distal end are several grooves which extend approximately 1 cm. into the bone over a length of about 2 cm.; the lower end of the bone is definitely heavy as compared with the upper end. The appearance is certainly most suggestive of a chronic infection, and the most likely diagnosis is syphilis.

2. *Left Femur* (fig. 2).—This bone weighs 298.3 Gm. and is 45.6 cm. in length. The angle of the neck is normal. There appears to be an anteversion of the head of about 5 degrees. The outstanding pathologic changes are found on the medial aspect of the bone, in its middle third and extending back to the posterior ridge, where there is an area of proliferative periostitis with bone plaques. The entire reaction is apparently periosteal and is most suggestive of syphilis.

3. *Right Femur.*—The weight of this bone is 278 Gm.; the length, 43.5 cm. The head and neck are unusually small for the size of the shaft and the condyles. There are considerable irregularity and pitting on the anterior, medial and lateral surfaces of the upper half of the bone, extending up as far as the neck. In several areas the appearance is that of proliferative periostitis, suggestive of syphilis. The bone is destroyed in a great many areas about the anterior portion of the head and neck and the posterior intertrochanteric area. These areas of destruction are probably a result of postmortem changes. The lower end of the femur is of normal appearance.

4. *Left Femur.*—The weight of the femur is 242 Gm.; the length, 43 cm. The head and neck appear to be unusually small, with a definite increase in the angle between neck and shaft, which measures approximately 145 degrees. The shaft of the bone does not seem to be enlarged in its lower portion as did that of specimen 3. There is an anteversion of the head and neck of about 10 degrees. There are the same pitting and erosion over the surface of the femur as was noted in specimen 3; this extends from the intertrochanteric area down the whole length of the bone and is most marked on its medial aspect. There has been considerable postmortem destruction of bone about the posterior intertrochanteric area and about either side of the head. The lower end of the femur appears to be unusually small. The specimen is suggestive of syphilis.

5. *Right Femur.*—This bone weighs 204 Gm. and is 49 cm. long. The angle of the neck appears to be normal. The head and neck are unusually small. There is an anteversion of the head and neck of about 10 degrees. There are

pitting and slight erosion of the entire anterior surface, with a few evidences of periosteal reaction. This, however, is not so marked as in specimens 3 and 4. The specimen is suggestive of syphilis.

6. *Left Femur.*—The weight of the bone is 366.5 Gm.; the length, 49 cm. This is an unusually long and somewhat heavy bone with an anteversion of the head and neck of about 10 degrees. The bone is in an excellent state of preservation,

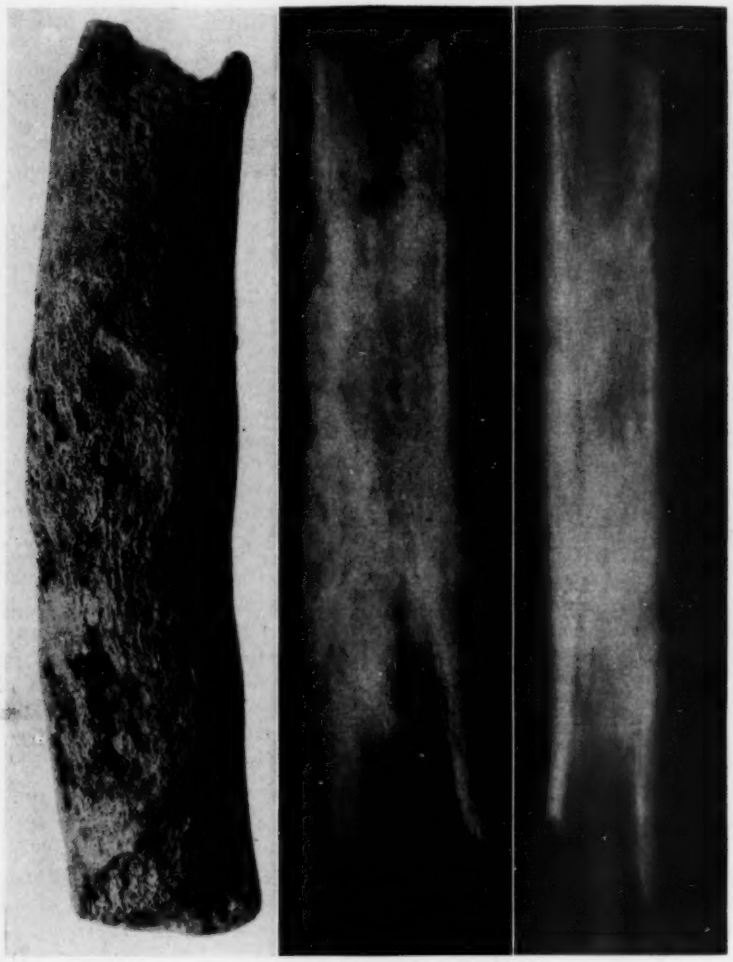

Fig. 3.—Photograph and roentgenograms of a section of a tibia, specimen 8. Note the erosion and anterior bowing in the photograph, also the periosteal proliferation, cortical thickening, moth-eaten appearance and partial obliteration of the medullary cavity in the roentgenograms. These changes are highly suggestive of syphilis.

there being no defects other than minute areas of broken bone around the greater trochanter. There is slight anterior bowing. Definite pathologic changes are

found in the lower third near the lateral condyle. There is definite proliferative periostitis, the surface being irregular, pitted and detachable from the underlying cortical bone. This area, however, is not so well defined as in specimen 2. Syphilis is the most probable diagnosis.

7. *Right Femur.*—The weight of this bone is 236.6 Gm.; the length, 43.75 cm. There is a slight decrease in the angle between the neck and the shaft, and the

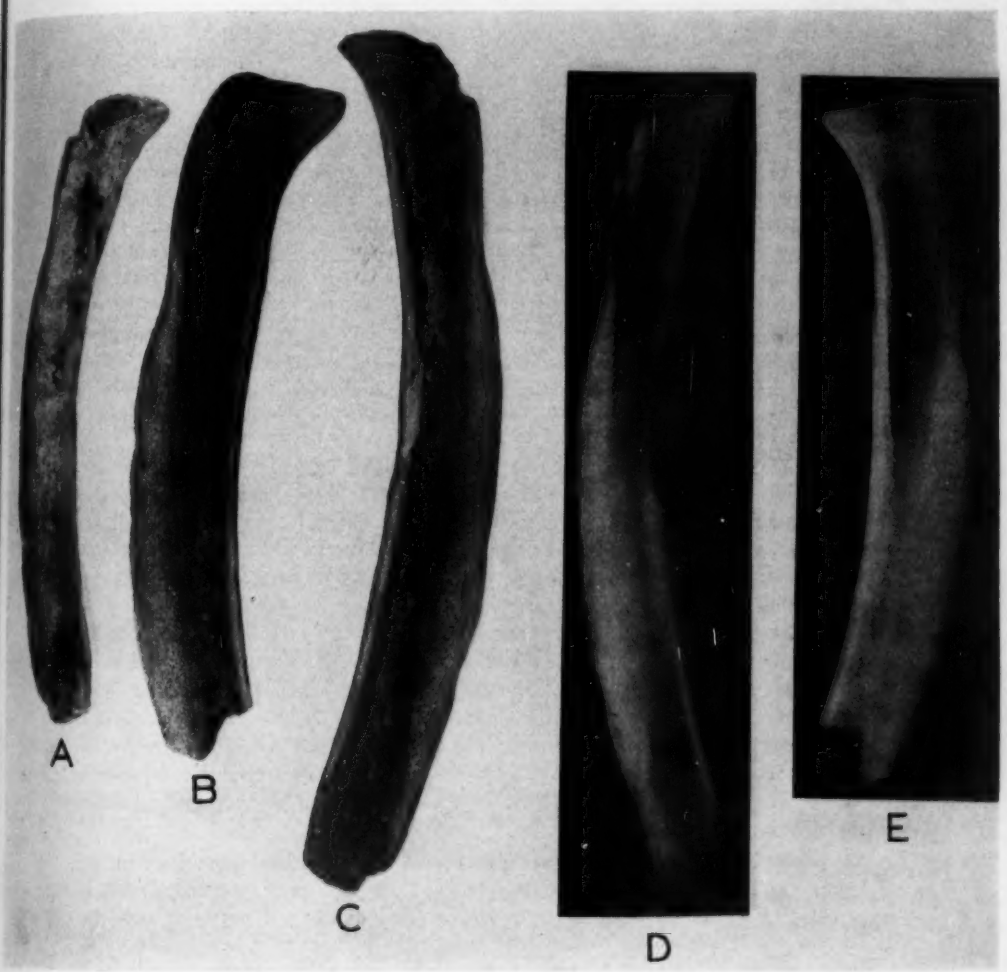

Fig. 4.—Photographs and roentgenograms of a tibia, specimen 9. *A* shows anterior bowing and cortical thickening. *B* and *C* are sections of the tibia which have been made by cutting the bone in its sagittal plane with a motor saw. Note the extreme thickening of the anterior cortex, the anterior bowing and the partial obliteration of the medullary cavity. *D* and *E* are roentgenograms of *C* and *B*, respectively, and show the same pathologic changes. The gross pathologic picture certainly could be a result of bone syphilis.

greater trochanter is unusually prominent. About the head are many areas of destruction, due to postmortem changes. There are rounding of the middle third of the shaft and slight anterior bowing, which may be due to osteitis deformans (Paget's disease).

8. *Middle Portion of Tibia* (fig. 3).—The bone weighs 147.3 Gm. and is 22 cm. long. This is the most striking specimen of the whole collection. It shows a large amount of cortical thickening as well as proliferation of the periosteum. There are numerous irregular areas and indentations over the whole anterior surface of the bone, some of which may be due to postmortem changes. The bone is unusually heavy. The cortex is extremely thick, its encroachment on the medullary cavity being visible at the broken ends of the bone. The changes are probably a result of syphilis or of chronic pyogenic osteomyelitis.

9. *Tibia* (fig. 4).—This specimen is in a poor state of preservation. The lower end of the bone is missing, and a large amount of damage has been done to the upper portion, the condylar surfaces having been broken off. There is marked anterior bowing, extending from the upper portion of the middle third down throughout the lower third. There are extensive thickening of the anterior portion of the cortex and evidences of proliferation of the periosteum. A sagittal section of the bone, made with a motor saw, shows that the anterior portion of its cortex is approximately three times as thick as normal. The periosteal proliferation can be seen to possess a well demarcated margin. The cancellous bone about the head of the tibia is friable. A large amount of dirt has accumulataed throughout the cancellous areas. This is an exceptional specimen and demonstrates clearly the chronic bone reaction attributed to syphilis.

10. *Right Tibia.*—This specimen weighs 132.2 Gm. and is 32.5 cm. long. It shows definite flattening of its condylar surfaces. There is a slight amount of anterior bowing in the middle and upper thirds. There is extensive new bone proliferation, including thickening of the cortex and periosteal reaction over the medial surface of the upper two thirds of the bone. This bone reaction may be due to syphilis.

11. *Right Tibia.*—This bone weighs 135.6 Gm. and is 37.5 cm. long. The surface of the lower two thirds is extremely irregular. There are areas of proliferative periostitis over the posterior aspect of the lower half. There is a moderate amount of pitting with small areas of erosion. The lightness of this bone suggests that it is from a very old person. The appearance is suggestive of syphilis, because of the periosteal reaction, but the defects may be due to pyogenic infection. If the lesions are syphilitic they are probably due to the acquired form of the disease.

12. *Right Tibia* (fig. 5).—The weight of the tibia is 333.6 Gm.; the length, 37.5 cm. This specimen is in a relatively good state of preservation. There is some chipping of the condylar surfaces and of the anterior and posterior surfaces of the lower end of the bone. The bone appears to be unusually heavy. There is a slight amount of anterior bowing of the upper and middle thirds. There is a large amount of cortical thickening over the anterior surface of this bone, especially on its medial and lateral aspects. The surface shows a moderate amount of pitting but no areas of deep erosion. The appearance is most suggestive of syphilis, probably of congenital type.

13. *Portion of Tibia.*—The bone weighs 111.2 Gm. and is 28.75 cm. long. This tibial fragment shows about its anterior and lateral aspects, areas of new bone formation, which are thought to have been caused by periostitis. There are

numerous irregular areas which show pitting and indentation; these may be post-mortem changes. The lesions undoubtedly represent chronic inflammation, which was probably the result of syphilis or of pyogenic osteomyelitis.

14. *Left Tibia.*—This specimen weighs 117.4 Gm. and is 31.5 cm. in length. There is moderate anterior bowing of the middle third, with thickening of the cortex about the medial aspect. There is evidence of periosteal reaction with irregularity and indentation of the anterior aspect of the lower third. The changes

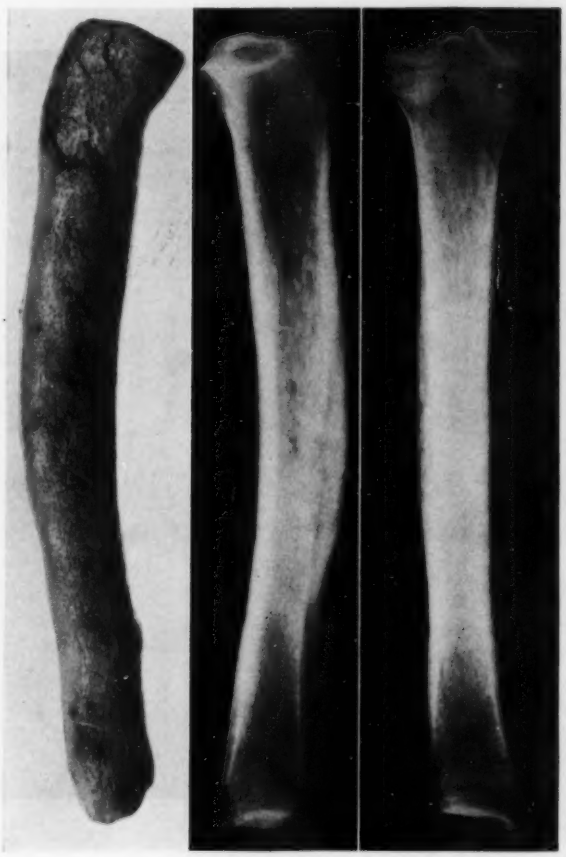

Fig. 5.—Photograph and roentgenograms of a tibia, specimen 12. The anterior bowing and cortical thickening are characteristic of syphilis, although pyogenic osteomyelitis is also to be considered.

are suggestive of a syphilitic reaction, although pyogenic osteomyelitis is to be considered.

15. *Left Tibia.*—The weight is 242.4 Gm.; the length, 39 cm. This specimen shows thickening of the upper two thirds of its posterior surface. There is proliferative periostitis with many irregularities and ridges throughout this upper portion. The appearance is suggestive of a saber shin. The changes may be due to syphilis or to pyogenic infection.

16. *Tibia.*—The bone weighs 298.3 Gm. and is 45.6 cm. long. The lower third of this specimen shows numerous areas of proliferation of the periosteum and new bone formation. On the anterior surface are several indentations which extend quite deeply into the cortex. Two of these seem to be connected by a sinus beneath the cortex. At one time these undoubtedly were draining bone sinuses. Areas of thickened cortex with new bone formation are present. The changes are due to localized osteomyelitis, which may be syphilitic or pyogenic in origin.

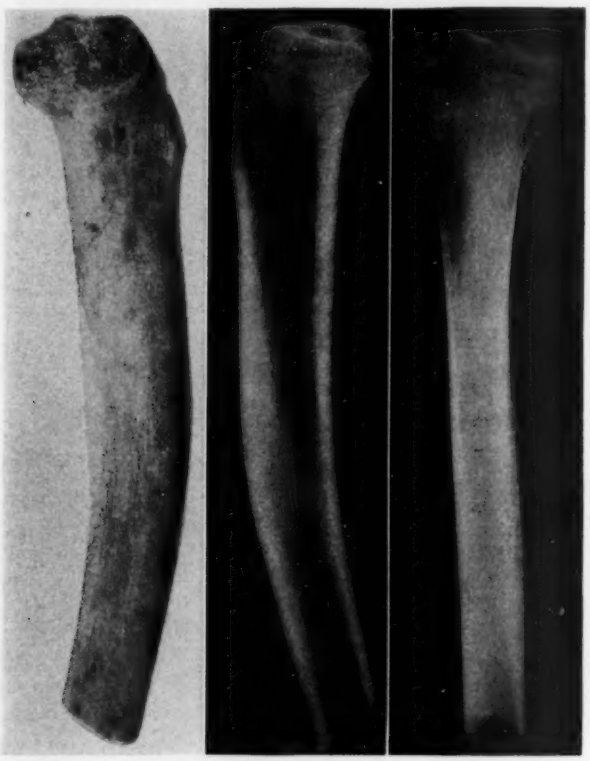

Fig. 6.—Photograph and roentgenograms of a tibia, specimen 17. Note the smooth outer cortex, the anterior bowing and the anterior cortical thickening. These changes are most suggestive of osteitis deformans (Paget's disease) but might be due to congenital syphilis.

17. *Left Tibia* (fig. 6).—The weight is 193.5 Gm.; the length is 32 cm. This specimen is unusually heavy. There is moderate anterior bowing of the middle third. There is a tremendous amount of thickening of the cortex on the medial aspect without marked irregularity of the surface. The changes may be due to osteitis deformans (Paget's disease) or to congenital syphilis.

18. *Left Tibia.*—The specimen weighs 241.3 Gm. and is 38 cm. long. There is a marked saber shin deformity, which extends from the tibial tuberosity to the

upper portion of the lower third of the shaft. There is no anterior bowing, but there is a slight amount of internal torsion. The surface of the cortex appears smooth and normal. There is a distinct narrowing of the external condyle of the tibia. The condylar surfaces do not appear to be as concave as normal, but seem flattened. It is most likely that some developmental change has taken place in the region of the upper epiphysis. This type of the saber shin is more characteristic of rickets than of congenital syphilis.

19. *Right Tibia.*—The weight of this bone is 175.5 Gm.; the length, 37.5 cm. There is a saber shin deformity, which extends from the tuberosity to the upper portion of the lower third of the shaft but which is not so marked as that of

Fig. 7.—Photograph of a skull. Note the deep stellate scars of Virchow and the areas of erosion. Of the entire collection, this specimen is the most typical of syphilis.

specimen 18. The cortex appears to be somewhat flattened. The medial condyle occupies a position considerably posterior to that of the external condyle. In this specimen, as in 18, there is a slight amount of internal torsion. These changes are suggestive of rickets.

20. *Right Tibia.*—The bone weighs 118.1 Gm. and is 37.5 cm. long. It shows a saber shin deformity, but this is not so marked as that of 18 and 19. It extends over a half of the anterior surface of the bone. The bone is extremely light as compared with the other tibias. The superior articular surfaces are definitely flattened. All internal torsion of approximately 10 degrees is present. The changes are probably due to rickets.

21. *Portion of Fibula.*—The weight is 30.5 Gm.; the length, 23.3 cm. The upper portion of this specimen shows a thickened cortex with evidence of proliferative periostitis. The cortex of the lower portion is apparently normal. The changes are suggestive of syphilis.

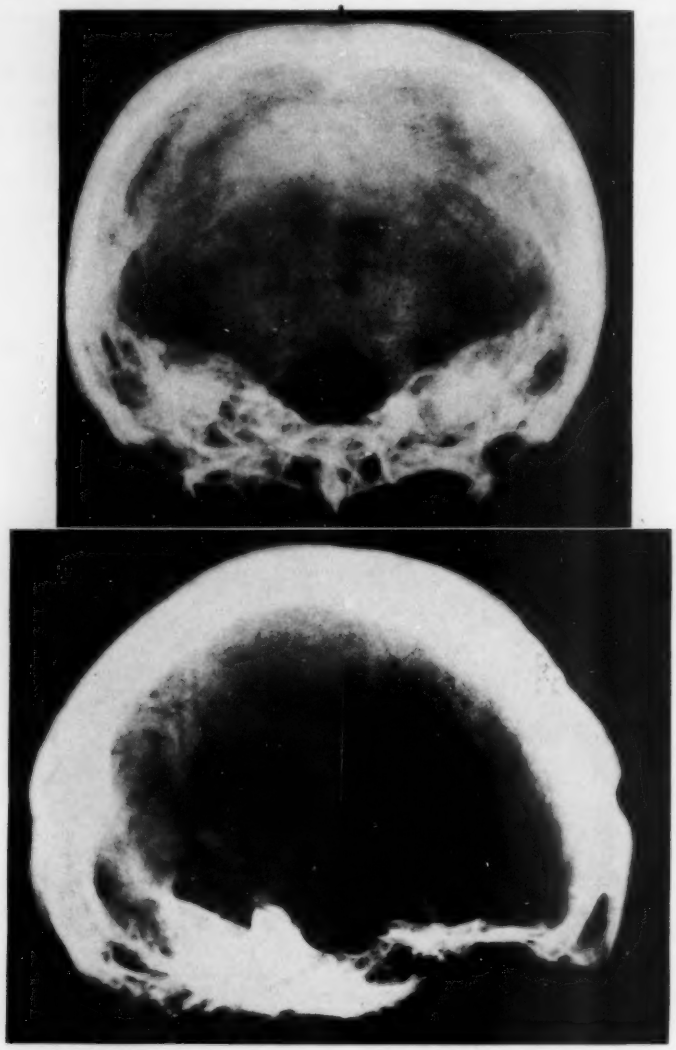

Fig. 8.—Anteroposterior and lateral roentgenograms of the skull shown in figure 7. Note the extreme thickening of the cortex in the lateral view and the irregular areas of absorption and proliferation of bone in the anteroposterior view.

22. *Fibula.*—The weight is 44.6 Gm.; the length, 33 cm. This specimen is in an excellent state of preservation. Over the lower portion of its upper half there is an area of periosteal bone proliferation with thickening of the cortex.

The thick new bone is fairly regular and appears to be definitely of periosteal origin. The changes may be due to an injury of the periosteum or to syphilis.

23. *Radius.*—The bone weighs 24.3 Gm. and is 20.5 cm. long. The lower half of this bone shows marked cortical thickening and periosteal reaction. The surface is irregular and marked by areas of erosion. The appearance is that of a chronic bone reaction which may be due to either pyogenic osteomyelitis or syphilis.

24. *Skull* (figs 7 and 8).—On the frontal bone there are stellate scars similar to those described by Virchow and marked by deep excavations presumably resulting from gummas. There is evidence of proliferation of new bone, the borders of the excavations being elevated above the surrounding surfaces. This specimen was examined by Dr. H. U. Williams of Buffalo, the foremost American authority on Indian bones, who states: "In my opinion the skull is in all human probability a syphilitic skull."

COMMENT

An analysis of these gross pathologic observations (table) shows that the only specimen which seems unquestionably to be syphilitic is the skull (fig. 7). This specimen shows admirably in the frontal bone the

Analysis of Gross Pathologic Observations on Bone Specimens

Diagnosis	Number	Percentage
Syphilis	1	4
Syphilis (with some question)	7	29
Syphilis or pyogenic osteomyelitis	10	42
Syphilis or osteitis deformans	2	8
Syphilis or trauma	1	4
Rickets	3	13
	24	100

stellate scars of Virchow so characteristic of syphilis. The roentgenograms of this skull (fig. 8) show great thickening of the cortex, which even made satisfactory roentgenograms difficult to obtain.

The three femurs, three tibias and one fibula which are probably syphilitic show extensive proliferation of the periosteum. These specimens and their roentgenograms (figs. 4 and 5) present the marked thickening of the cortex which is characteristic of syphilis. This thickening is evident in the sagittal section of the tibia illustrated in figure 4. These specimens are similar to the ones reported by Means.[1]

The ten specimens which exhibit the lesions either of syphilis or of pyogenic osteomyelitis present various degrees of periosteal proliferation and cortical thickening. In the roentgenograms the thickening is not so marked, as that of the specimens in the previous group. The localized involvement of the lower part of a tibia is rather characteristic of a pyogenic bone reaction but is occasionally seen in syphilis. The localized periosteal reaction of the specimen shown in figure 2 is quite characteristic of an early syphilitic bone reaction.

1. Means, H. J.: Am. J. Roentgenol. **13**:359, 1925.

The femur specimen 77 and the tibia specimen 17 shown in figure 6 suggest osteitis deformans. The tibia shows anterior bowing and a greatly thickened anterior cortex. Although these lesions are sometimes found in congenital syphilis, the absence of periosteal changes is against syphilis.

The three rachitic tibias are very typical. Each has a smooth cortex with the narrow anterior margin so characteristic of a saber shin but show very little anterior bowing. These findings could, however, be due to congenital syphilis.

There appears to be sufficient evidence in these twenty-four bone specimens to prove that syphilis existed in the Mound Builders many centuries before the discovery of America by Columbus.

The most complete presentation of this subject has been made by Dr. Hubert U. Williams,[2] of Buffalo, in his article entitled "The Origin and Antiquity of Syphilis: The Evidence from Diseased Bones," which was published in 1932 in the ARCHIVES OF PATHOLOGY.

SUMMARY

Archeological excavations and findings in the mounds at Moundsville, Ala., are reviewed. Gross pathologic changes found in twenty-four bone specimens are described. In twenty-one of these the diagnosis of syphilis is suggested. Photographs and roentgenograms of eight of the most typical bone specimens, the majority of which show changes characteristic of syphilis, are presented.

2. Williams, H. U.: Arch. Path. **13**:779, 1932.

Case Reports

LEIOMYOSARCOMA OF THE URINARY BLADDER

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Sarcoma of the bladder is not common. The number of reported cases is difficult to determine. Cecil [1] in 1926 stated that there were 193. Six years later, Gabe [2] said that the number exceeded 130. This confusion applies not only to the number of reported cases but to their histologic classification and results from the fact that many of the earlier reports are inadequate in giving details and that some reviewers of the literature failed to give specific references to the literature for the instances collected.

Leiomyosarcoma comprises one of the smallest histologic classes of malignant tumors of the bladder. A review of the literature showed 4 authentic cases. The first case was reported by Röder,[3] the second by Hager and Hunt,[4] the third by Caylor and Walters [5] and the fourth by Krauskopf [6] (table). The sarcoma reported by Eve [7] is listed by Albarran [8] as myosarcoma and by Wilder [9] as spindle cell and round cell sarcoma. Eve was not sure of its classification; his own diagnosis was "mixed-celled and probably myosarcoma." Eve's histologic description, though given in great detail, is of little help since he did not have the advantage of differential muscle fiber stains (the Van Gieson stain was introduced in 1896). An additional case was found in which leiomyosarcomatous elements were mixed with malignant epithelial cells. This case was recorded by Gussenbauer.[10] Nicolich [11] and Lexer [12] each reported a case of myosarcoma of the bladder but did not state the muscle type or give histologic descriptions of the tumors. The other reported that malignant muscle tumors of the bladder were either entirely rhabdomyomatous or of a mixed nature.

From the National Institute of Health, Washington, D. C., and the United States Marine Hospital, Baltimore.

1. Cecil, H. L.: J. Urol. **16**:473, 1926.
2. Gabe, J.: Brit. J. Urol. **4**:145, 1932.
3. Röder: Deutsche med. Wchnschr. **30**:485, 1904.
4. Hager, B. H., and Hunt, V. C.: J. Urol. **21**:129, 1929.
5. Caylor, H. D., and Walters, W.: J. Urol. **24**:303, 1930.
6. Krauskopf, H.: Am. J. Obst. & Gynec. **24**:133, 1932.
7. Eve, F. S.: Tr. Path. Soc. London **36**:284, 1885.
8. Albarran, J.: Les tumeurs de la vessie, Havre, Lemale & Cie, 1891.
9. Wilder, J. A.: Am. J. M. Sc. **129**:63, 1905.
10. Gussenbauer, C.: Arch. f. klin. Chir. **18**:411, 1875.
11. Nicolich, G.: Riv. veneta di sc. med. **11**:334, 1889.
12. Lexer: Deutsche med. Wchnschr. **30**:42, 1904.

Pertinent Data on Four Valid Cases of Leiomyosarcoma of the Urinary Bladder

Observer	Patient's Age and Sex	Symptoms	Location of Tumor	Gross Appearance	Microscopic Appearance	Operation	Metastases	Outcome
Röder [3]	49 M	Intermittent pain for 4 years; loss of weight	Not definitely stated, probably right lateral wall	Irregularly oval, 15 by 8 by 7 cm.; reddish yellow; nodular surface protrusions; white cut surface; focal necrosis; invasion of ureter	Leiomyosarcoma; cells recognizable by their rod-shaped nuclei and by the intense affinity of the cytoplasm to trinitrophenol; many cells atypical	Resection of tumor with portion of bladder wall	None	Patient died 5 days after operation of peritonitis; autopsy showed peritonitis and pyonephrosis
Hager and Hunt [4]	53 F	Hematuria for 10 days prior to admission; little pain; urine contained albumin 3+, pus 4+, blood 2+	Posterior wall	Truncated cone shape, 2 by 2.5 by 2.5 cm.; smooth surface; small area of ulceration	Leiomyosarcoma, grade 1; intertwining nonstriated muscle fibers in areas interspersed with connective tissue fibers; nuclei conspicuous; few mitotic figures	Resection	No clinical evidence of metastasis	Patient had uneventful recovery and left hospital 24 days after operation; no follow-up data
Caylor and Walters [5]	4 M	Frequency, incontinence and dysuria for 8 mo.; nocturia for 5 mo.; little hematuria; residual urine; blood 2+; 45 pus cells per low power field	Two tumors; larger one on right side of dome; second on anterior wall	(1) pedunculated, 7.5 cm. in diameter; (2) partly pedunculated, 4 by 3 by 3 cm.; grayish white, slightly irregular surface; hemorrhage in smaller tumor	Large cells, resembling smooth muscle cells, arranged in bundles in few areas; some large cells had multiple nuclei; bladder wall invaded	(1) Cautery removal (2) resection	No clinical evidence of metastasis	Diffuse recurrence in base and anterior wall 2 mo. after operation, treated by radium; patient died 1 mo. later; no autopsy
Krauskopf [6]	60 F	Dysuria and frequency for 2 mo.; loss of weight and enlargement of abdomen past 6 weeks	Anterior wall; growth almost filled cavity of bladder	Well encapsulated at all points; bladder wall ¾ in. thick, (diffuse and flat tumor?) 18 by 9 by 3.6 cm.; surface irregular, with some superficial and deep notches; bladder and tumor weighed 420 Gm.	Mature and immature muscle cells; others with hyaline and mucoid degeneration; mitoses abundant; few connective tissue fibers	Resection	No clinical evidence of metastasis	Death 39 days after operation following sudden suppression of urine; no autopsy

The case reported now is that of a pure (unmixed) leiomyosarcoma and is the fifth authentic case of malignant smooth muscle tumor of the urinary bladder.

REPORT OF CASE

A white man aged 59 years was admitted to the United States Marine Hospital, Baltimore, Nov. 19, 1936, with urinary retention and pain in the region of the bladder. The family history was irrelevant.

The patient had amebiasis from 1904 to 1911, which recurred for a short period in 1912. Since this illness he had only partial control of the rectum. While he was working under compressed air in 1923, his legs became partially paralyzed, and he was unable to urinate. These symptoms recurred in November 1933, at which time cystoscopy showed marked suppurative cystitis. A cystogram and a pyelogram showed a large diverticulum of the left lateral wall of the bladder, a small one on the right and downward compression of the upper calices of the right kidney (probably due to rotation of the kidney). Diverticulectomy and bilateral vasectomy were performed in December 1933 and suprapubic prostatectomy in January 1934. Microscopic examination of the diverticula showed degenerative changes involving the musculature. The prostate showed adenomatoid hyperplasia with subacute and chronic prostatitis. An x-ray picture showed fibrous proliferation in the upper lobes of both lungs and interlobar pleurisy of the right lung. The roentgenologic impression was: tuberculosis. On July 31, 1934, the patient was improved but still troubled with cystitis.

On admission to the hospital he complained of pain in the region of the bladder, occasional inability to urinate, smallness of the urinary stream, the voidance of "masses of pus," nocturia, very slight burning and urgency and loss of 15 pounds (6.8 Kg.) in the past year. There was no history of hematuria. There had been urinary retention for a few days in September 1936. The patient stated that his bladder trouble began in 1923, when he had partial paralysis of the legs and was unable to void urine. The dates and the order of occurrence of the aforementioned symptoms were not recorded.

Physical examination following relief of acute retention of urine showed marked tenderness in the region of the bladder on deep abdominal pressure and rectal examination. No tumor was palpated; the prostate was absent. The temperature, blood pressure, heart and lungs were normal; the knee jerks were hyperactive. Cystoscopy was unsatisfactory because of the presence of much débris in the bladder; there was marked cystitis, but the tumor was not seen. Excessive mobility of right kidney and hydronephrosis on the left were noted in the pyelogram. An x-ray picture of the chest showed bilateral thickening of the apical pleura and considerable infiltration of the lower right pulmonary field. An x-ray picture of the spine showed marked destruction of the tenth dorsal vertebra with ankylosis (an injury of the back occurred in 1927). Subsequent x-ray pictures of the chest showed slowly resolving pneumonia. There was slight anemia, and repeated urinalysis revealed a variable quantity of albumin and pus cells.

On Dec. 18, 1936, through a suprapubic cystostomy opening a specimen was removed from a large flattened tumor mass which occupied the left side of the bladder. The tumor was considered inoperable. The histologic appearance of the growth was the same as described in the next paragraph for the specimen obtained at autopsy. Following the cystostomy the patient had considerable cough, pain in the region of the bladder and a daily septic rise of the temperature; he became progressively weaker and died Jan. 15, 1937.

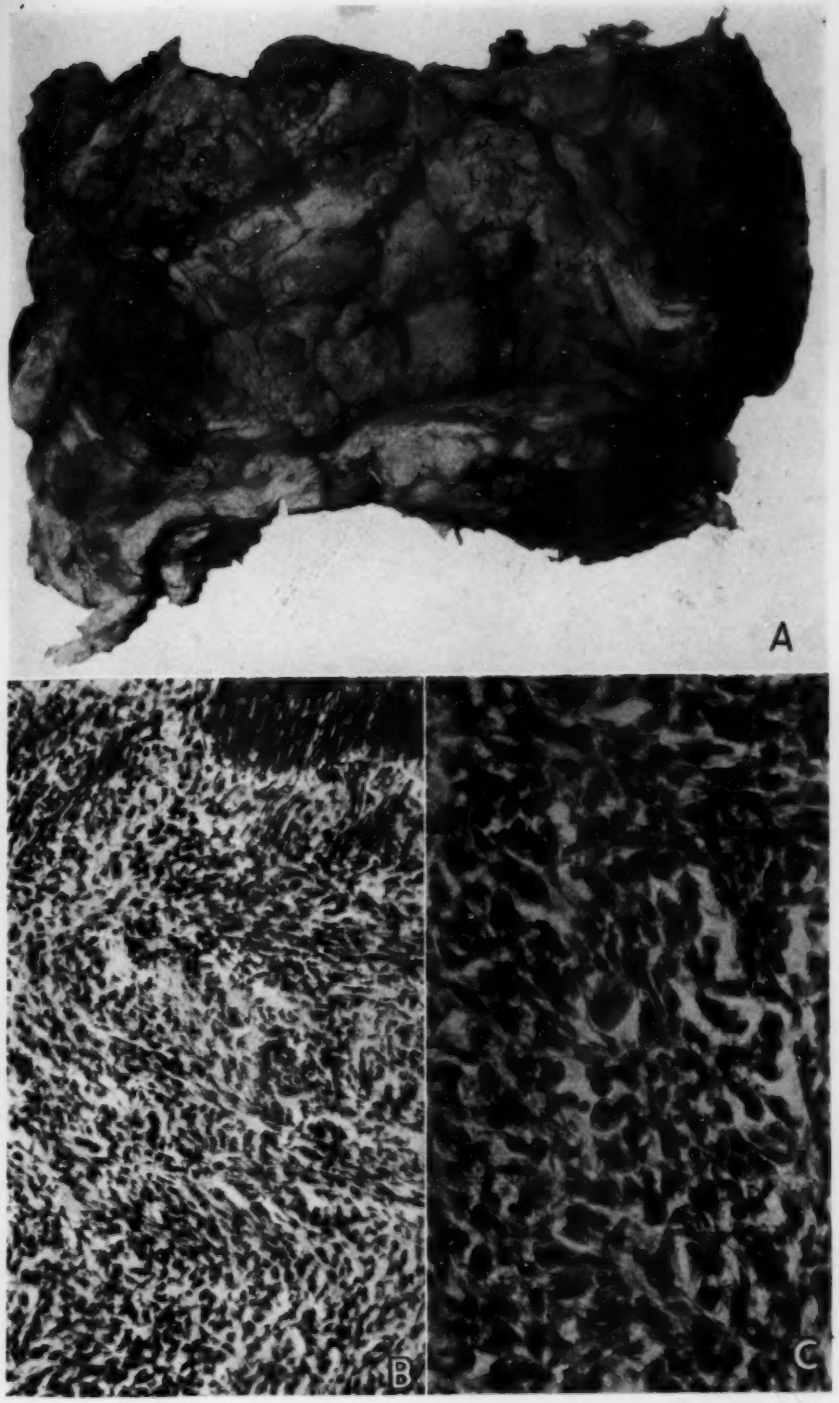
Necropsy.—The observations included: a tumor of the bladder (to be described), cystitis, severe bilateral ureteritis, bilateral pyelonephritis, bilateral adhesive fibrous pleurisy of the lower lobes of the lungs, a cavity of the lower lobe of the right lung, emaciation, chronic passive congestion of the viscera, arteriosclerosis—general and cerebral—and decubital ulcers of the back. No metastatic deposits were found.

A large diffuse tumor mass, flattened and superficially necrotic, covered about three fifths of the inner surface of the bladder. The anterior wall, the anterior part of the right lateral wall and the anterior portion of the trigon were not involved. Both ureters traversed the tumor, were narrowed but not completely obstructed. The surface of the tumor showed raised, irregularly sized nodular masses giving a cauliflower appearance. On section the tumor was yellowish white and firm and invaded the wall of the bladder for varying depths. At no point did it involve the entire thickness of the wall. The thinnest part of the tumor was on the left lateral wall near the urethral orifice. Much necrotic débris was present in the cavity of the bladder.

Microscopic Examination.—A broad, flattened tumor mass replaced the mucosa and invaded the muscularis. The bladder epithelium was lost at the margin of the tumor in the necrotic mass which involved the free margin for varying depths. The uninvolved wall of the bladder was 7 mm. thick, and peripheral to the tumor it measured 1.5 mm. and showed considerable interstitial scarring and some atrophy of muscle. The tumor cells were of medium to large size, usually fusiform and, in many areas, roughly arranged in fascicles. Delicate intercellular collagen fibers were present in most areas, and thicker, ramifying fibrous bands occurred, diffusely infiltrated by tumor cells. The nuclei were very hyperchromatic; they were vesicular and varied in diameter up to 22 microns. These larger cells were in the minority and were scattered, though fairly numerous in a few areas. Multiple or lobate nuclei with large nucleoli were fairly common, and mitoses were moderately numerous. Cellular cytoplasm was variable in quantity, stained deep yellow with Van Gieson's solution of trinitrophenol and acid fuchsin and frequently showed distinct, longitudinally arranged parallel threads (myofibrillae —fig. 2 *B, C* and *D*). Medium-sized polygonal cells were moderately numerous only in edematous areas bordering on necrosis. Thin-walled vascular channels were intimately surrounded by tumor, but no definite invasion of a wall of a vessel was seen. The invading margin of the tumor was convex and fairly smooth, though short projections occurred between muscle bundles. Bundles of preexisting smooth muscle were present deep in the tumor mass, but no transition was seen. Eosinophils, lymphocytes and fewer neutrophils were present, usually in superficial areas.

COMMENT

In previous reviews and discussions of sarcoma of the bladder, conclusions regarding the symptoms, location and clinical behavior have been based on observations of the group as a whole, including the various cell types. It does not appear reasonable to expect that leiomyosarcoma and lymphosarcoma have a similar clinical course simply because they originate in the same organ. The inclusion of cytologically distinct tumors in the same group for the purpose of statistical analysis probably explains the disagreement of some authors on various aspects of the disease. Two main points of disagreement are the questions of site of origin and frequency of metastasis. With reference to the latter Albarran [8] stated that metastases occurred in 13.2 per cent of his col-



EXPLANATION OF FIGURE 1

A, gross photograph of the bladder and tumor with the anterior wall of the bladder removed; the trigon is at the right; × 2/3.

B, old muscle fibers in the upper right corner. Note the fairly prominent fasciculation. Masson's trichrome stain; × 125.

C, higher magnification of tumor cells. Note mitoses in scattered cells. Van Gieson stain; × 300.

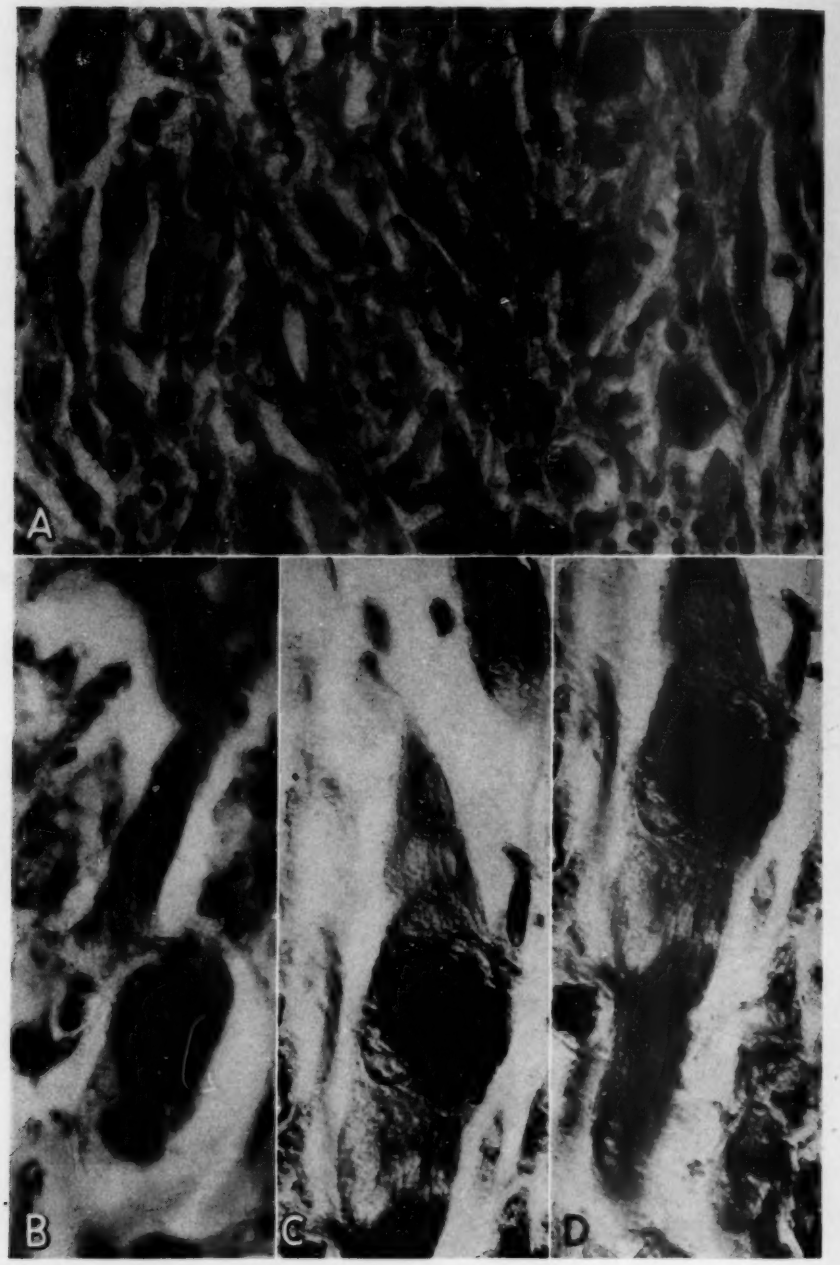


Fig. 2.—*A* shows many tumor giant cells. Note their fusiform nature and the tapering extremities of the smaller cells. Van Gieson stain; × 380.

B, *C* and *D* show large cells with myofibrillae. *C* and *D* show opposite ends of the same cell. Phosphotungstic acid hematoxylin stain; × 750.

lected instances; Cecil,[1] 21.7 per cent, and Munwes,[13] 52.2 per cent, whereas Concetti [14] found metastases only once in 42 cases, and Mixter [15] stated, "They do not tend to metastasize." The opinions of Concetti and Mixter are based on sarcoma of the bladder in children, and the figures given by Albarran, Cecil and Munwes relate to cases in persons of widely varying ages. This confusion will quite likely continue until case analyses are confined to a grouping in which the individual group contains tumors of one cell type only. At the present time division of sarcoma of the bladder will result in some groups too small to justify general conclusions. The case presented here falls into such a group, since only 5 cases of leiomyosarcoma have been reported. However, the pertinent data concerning these cases are recorded in the accompanying table (present case not included) for the purpose of initiating the division referred to, since we believe that this group will soon be large enough to allow trustworthy statistical analysis. This belief is based on the fact that previous to 1929 only 1 case was reported and since then 4 cases have been found. There is nothing to suggest that these tumors are becoming more frequent. A more probable explanation of the larger number is the slowly but surely increasing usage of multiple and special stains in the diagnosis of tumors.

Figures 1 and 2 show the gross and microscopic pathologic character of the tumor reported here and indicate the stains used in arriving at the histologic diagnosis.

SUMMARY

A case of leiomyosarcoma of the urinary bladder is presented together with brief summaries of the previously reported cases. No comparative study is made since only 5 tumors of this type have been recorded.

13. Munwes, C.: Ztschr. f. Urol. **4**:837, 1910.
14. Concetti, L.: Boll. d. r. Accad. med. di Roma **26**:271, 1900.
15. Mixter, C. G.: Ann. Surg. **65**:628, 1917.

ENDOCARDIAL POCKETS

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AND
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Among the interesting pathologic changes encountered in routine postmortem studies is a rare structural abnormality which is of questionable or unknown clinical importance. This is the endocardial pocket which, although probably nonproductive of clinical phenomena, is of singular interest.

Recently, we observed this structure in three cases. We are reporting these cases in the hope that we may add corroborative evidence of the factors responsible for the formation of such pockets.

The excellent study of Saphir [1] is one of few reports in the American literature on this subject and has provided much of the information on which this brief study is based.

The endocardial pocket, or pseudovalve, is found on the interventricular or interauricular septum in a heart which shows insufficient mitral or aortic valves. It is not found in all cases in which insufficient valves are present. It may be overlooked easily or may not be recorded; possibly the incidence of such pockets is higher than the few reported cases would indicate. The endocardial pocket consists of a fold of thickened endocardium and usually is of a rough semilunar contour. The opening of the pseudovalve may be toward the aorta; this opening has been named the "diastolic pocket" by Krasso, and that which opens toward the apex of the heart he has designated the "systolic pocket."

Various theories have been propounded to explain the pocket etiologically. The first and most acceptable one is that the pocket is the result of the mechanical irritation caused by the constant force of regurgitance of blood through an insufficient valve. This theory has been rejected by some authors, but there are many points in its favor. Perhaps the best reason for believing that the endocardial pocket is formed on a mechanical basis is that it occurs only in association with a defective cardiac valve. One investigator pointed out that its

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From the Section on Pathologic Anatomy, the Mayo Clinic.

1. Saphir, Otto: Am. J. Path. **6**:733, 1930.

presence is often an aid in establishing the diagnosis of incompetence of the valve. Its formation on a mechanical basis would not exclude the possibility of a primary inflammation followed by the hollowing out process resultant from regurgitation of the stream of blood. Thus, the second theory becomes evident; that is, the pocket is due to an inflammatory process in the endocardium. Krasso [2] said that an infected thrombus may form the background for the development of the pocket, but the mechanical factor must also be present to complete the formation. It seems incredible that endocarditis could remain localized to one small region such as that occupied by such a structure. The belief has been expressed that a "valve" of this type is evidence of functional adaptation. This theory is hardly acceptable because very often the capacity of the pocket is almost negligible. It has been suggested that the pocket is a congenital malformation. The evidence in proof of this is not conclusive, yet its application cannot be excluded. There may be congenital folds of endocardium which require only the hemohydraulic factor of the regurgitation resulting from insufficiency of the valves to form pockets or pseudovalves.

Microscopic examination of such structures has proved instructive. Some sections have shown only hyalinization with few nuclear remnants, but in other sections evidence of a recent inflammatory process was found.

In the three cases which form the basis of this study portions of each pseudovalve and the surrounding endocardium were studied microscopically.

REPORT OF CASES

CASE 1.—The patient was a woman aged 31 years. The heart weighed 380 Gm. The leaflet of the mitral valve, particularly the free margin, was rolled and thickened. The orifice was stenotic, and the chordae tendineae were thickened and shortened. Along the free edges of the cusps of the aortic valve, there were small firm white vegetations. In addition to these, there were large friable hemorrhagic verrucous vegetations on the cusps. Some of these vegetations extended downward on the wall of the septum and the chordae tendineae of the posterior papillary muscle; 5 cm. below the aortic ring, on the interventricular septum, the endocardium was thickened. In this thickened endocardium there was a fold of semilunar contour which measured 0.8 cm. across its opening (fig. 1). It had the appearance of a valve, but the capacity was negligible. The opening of this pocket was toward the aortic cusps. The endocardium composing it, also the adjacent tissue, showed no macroscopic evidence of recent inflammatory change. The pulmonic and tricuspid valves were normal. There was slight sclerosis of the coronary arteries.

Microscopically, this fold of endocardium (fig. 2) was composed of the loose form of adult connective tissue peculiar to normal heart valves. The subendocardial elastic tissue split to form laminae on both the inner and the outer surface of the pseudovalve and also provided a fine elastic meshwork for the connective

2. Krasso, Hugo: Frankfurt. Ztschr. f. Path. **37**:136, 1929.

Fig. 1.—Diastolic endocardial pocket in case 1.

Fig. 2.—Microscopic appearance of a section of the endocardial pocket in case 1; the homogeneous substance on the left surface of the pseudovalve is gelatin, which was used to coat the gross specimen; hematoxylin and eosin; × 38.

tissue structure. Into the base of the pseudovalve, bundles of histologically normal heart muscle extended in much the same manner as muscle extends into the tricuspid valves of normal infants. Thickening of the endocardium was minimal. There was no evidence of inflammatory reaction at the base of this pocket, but a definite hyaline thickening of the endocardium was found about 0.6 cm. from the pocket. In short, this pseudovalve had every appearance of a normal young heart valve.

CASE 2.—The patient was a man aged 34 years. The heart weighed 429 Gm. There was marked stenosis of the mitral orifice (1 cm. in diameter) with the resulting fish mouth deformity. There was calcification in the leaflets of the mitral valves. The aortic valve was free from structural change. About 1 cm. below the base of the aortic valve, on the interventricular septum, there was a patch of thickened endocardium. In this area there were three semilunar puoches, whose openings were directed toward the aorta. The three pockets were situated one above the other, with a 0.5 cm. space between them. They were not connected, and each measured 0.5 cm. across the opening. They appeared as reduplications of the aortic cusps. There was practically no sclerosis of the coronary arteries.

Histologic examination of the sections of this pseudovalve proved that it was different from the pocket in case 1 in that there was a marked abrupt thickening of the endocardium at the base of the pockets, they contained no heart muscle bundles, and, most important of all, the base of the pockets and the subjacent myocardium were interspersed diffusely by polyblasts, lymphocytes and plasma cells. Marked congestion of capillaries and thickening of the walls of the terminal arterioles also were present in this region. The connective tissue composition of the valvelike structure, including the arrangement of elastic fibers, was essentially the same as that of the pocket in case 1. A patch of hyaline thickening of the adjacent endocardium was also present in the section.

CASE 3.—The patient was a man aged 58 years. The heart was tremendously hypertrophied and dilated. It weighed 1,170 Gm., including the pericardium, which could not be detached from the epicardium because of dense fibrous adhesions. The aortic valve and ring were extensively calcified, and the lumen of the aortic valve had undergone stenosis. About 1.5 cm. below the middle of the aortic cusp, on the endocardium of the interventricular septum, there was a pair of folds, semilunar in shape, whose openings were directed toward the apex of the heart (fig. 3). These pockets measured 1 cm. across the mouths, but there was little or no cavity found in them. The coronary arteries had undergone extensive sclerosis and calcification.

Histologic examination of the pseudovalve proved it to be similar to that in case 1 as there was no evidence of inflammation. The pockets had, however, a more compact connective tissue structure, and there was a definite relative increase in the number of nuclei. The endocardium in the region of the pockets was slightly thickened in several patches by compact connective tissue similar to that of the pockets. This connective tissue, however, was adult in type as it contained no elements which could be considered fibroblastic.

COMMENT

In these cases the endocardial pockets or pseudovalves occurred in hearts which showed structural changes resulting from (1) subacute bacterial endocarditis superimposed on old rheumatic endocarditis, (2) chronic rheumatic endocarditis and (3) calcareous aortic stenosis.

From the evidence obtained in these cases, it may be assumed that the most logical basis of these endocardial reduplications was the mechanical influence of the blood current which had been altered by valvular defects. Saphir discussed the importance of serial sections in the study of the anatomic features of this lesion, particularly in the search for foci of inflammation. It is possible that foci of inflammation might have been found by serial sectioning of the pockets in these cases, but it is evident that demonstration of foci of inflammation in itself does not prove either the inflammatory or the mechanical origin of these

Fig. 3.—Systolic endocardial pockets in case 3.

lesions. In the case in which marked inflammatory reaction was present there was no infectious basis for the inflammation so far as we know, but the factor of mechanical irritation as evidenced by valvular deformity was prominent. The pocket in which inflammation might have been expected, on the other hand, namely, that in the case of subacute bacterial endocarditis, proved particularly free from inflammatory reaction; in fact, the pseudovalve had a striking resemblance to the normal young cardiac valve. It was also striking in these cases that the endocardium in the region of the endocardial pockets was subject to an influence which had produced endocardial thickening.

If this thickening was a modification of the pocket, it was devoid of inflammation and subject to the only influence which had been brought to bear on the endocardium of all three hearts, namely, mechanical irritation from the altered course of the flow of blood, due to valvular defects. We freely admit that we have no evidence at hand to refute completely the theories of inflammatory, congenital or thrombotic origin of pseudovalves, but we do not believe that these theories of origin are as logical as the theory of mechanical irritation in view of the fact that the endocardium of the affected region is generally thickened in cases of valvular deficiency and there is no histologic evidence of previous thrombosis. We also are forced to admit that we have no explanation for the relative infrequency of endocardial pockets even in cases of valvular heart disease.

SUMMARY

For want of fuller explanation of the cause of these lesions, the theory of mechanical irritation of the endocardium caused by abnormal blood currents is accepted as the most logical.

General Review

RECENT CONTRIBUTIONS TO THE IMMUNOLOGY OF HELMINTHIC INFECTIONS

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(Concluded from page 117)

IV. APPLICATIONS OF IMMUNOLOGIC PHENOMENA IN HELMINTHOLOGY

Immunologic reactions have been used in helminthology primarily for two purposes: to classify the parasites both among themselves and in relation to other forms, and to reveal the presence of parasites in a host. The first application is made possible because with helminths as with all living forms relative proximity of relationship is shown by relative intensity of immune reactions when other antigens are substituted for the specific one used in preparing the antibody employed in the tests. Without these partial reactions with antigens from related organisms, immunologic tests could not be used for classifying any of the groups of living things. Yet this same character, which permits their use for taxonomic purposes, generally limits and often obscures their value in the diagnosis of infections. Nevertheless, such tests have already assumed a significant role with the diagnostician, and in some cases symptoms which defy explanation by any other means available are, through immunologic tests, revealed to be due to the presence of helminths.

CLASSIFICATION OF HELMINTHS (NECESSITY OF CROSS-REACTIONS)

Immunologic reactions have been extensively used in classifying many kinds of organisms but have had only relatively limited application as yet for this purpose among the helminths. While some evidence has been presented to indicate that morphologically related groups within the helminths are related antigenically (Fairley and Jasudasan; Fairley, 1931 and 1932), no systematic study on this point has as yet been

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carried sufficiently far to reveal whether or not the immunologic reactions will have critical influence in helminth taxonomy. An interesting paper by Eisenbrandt recently appeared in this field, however, which suggests what may be possible through immunologic studies. This author prepared antiserums against a number of helminths of widely separated taxonomic groups by injecting into rabbits the corresponding antigens. The following parasites were employed: (Nematoda) Toxocara canis, Ascaris suis and Ascaridia lineata; (Acanthocephala) Macracanthorhynchus hirudinaceus, and (Cestoda) Taenia pisiformis and Moniezia expansa. The results attained by precipitin tests generally paralleled the accepted systematic positions of the species tested, although it was indicated, as an exception, that the Acanthocephala were more closely related antigenically with the Platyhelminthes than with the Nemathelminthes.

It is as yet impossible to appreciate whether the proteins of the worms or the lipoids and carbohydrates, if present and functional antigenically, direct the specificity of the reactions which can be and have been demonstrated. Campbell has reported, however, that in Ascaris lumbricoides a polysaccharide occurs which in his experiments is antigenic, giving rise independently of the proteins of the ascarid to antibody on injection into rabbits. A large and as yet relatively unexplored field here awaits the immunologist.

DIAGNOSIS OF HELMINTHIC INFECTIONS (LIMITATION BECAUSE OF CROSS-REACTIONS)

All of the various modes of demonstrating immunologic reactions have been invoked in diagnosing helminthic infections. The complement fixation test, the precipitin test and the skin test have enjoyed the most extensive use. In acute helminthiasis, the worm materials can sometimes be demonstrated in the blood of the patient or of the infected animal, or, more commonly, the antibody can be detected in the host's serum a few days or weeks after infection by using material from the parasite as the antigen in the test. In general, the tests for antibodies do not become positive before the second or third week after infection, since time must elapse for the host to react against the invasion by the parasite. Furthermore, in order that the host may form antibody it must be actually affected by the parasite either through the latter's invasion of its tissues or through the parasite's secretion of some substance which is absorbed by the host. In general, parasites which dwell only in the lumen of the intestine stimulate only traces of antibody at best, and immunologic tests can be applied for diagnosing the presence of these forms with only limited success. In contrast, parasites which reach muscles, or those which have a blood stream passage, stimulate larger amounts of antibody, and infection by them can be identified with greater facility.

Preparation of Antigens.—The preparation of antigens for the diagnosis of the helminthiases by immunologic tests is a matter of the utmost importance and, at the same time, of considerable difficulty. The helminths are low in protein content, and as yet the immunologic significance of the lipoids and polysaccharides which they may contain is not understood. Extracts of the worms are usually made, sometimes following treatment with ether. The extractant used is generally physiologic solution of sodium chloride or Coca's solution. An alcoholic extract is frequently tried, the dissolved worm substance later being recovered and dissolved or suspended in salt solution. It must be admitted that as yet no standard method of preparing helminthic antigens has been arrived at; after a satisfactory method has been worked out, perhaps the immunologic tests will enjoy wider use for diagnostic purposes.

(*a*) Trematoda. Fairley and Jusadasan showed that an extract of the cercarias of any species of mammalian schistosome can be used as antigen to detect antibody called forth by infection with any mammalian schistosome. For his complement fixation tests, Fairley used an alcoholic extract of the fresh livers of snails, Planorbis exustus, heavily infected with Schistosomum spindale. Fairley and Jasudasan were unable to use the adult schistosomes with success for the purpose. Fairley and Williams, as well as the Taliaferros, used for skin tests a saline extract of infected snail livers which had been previously dried over calcium chloride. Khalil and Hassan found the snail antigen unsatisfactory for skin tests in schistosomiasis and substituted instead adults of Schistosomum bovis. Alcoholic and saline extracts of Schistosomum bovis have sometimes proved useful as antigen in complement fixation tests for schistosomiasis (Salam). But such extracts in skin tests have been replaced, in turn, by an extract of adults of Fasciola gigantica, which are much more readily available than those of Schistosomum bovis (Hassan and Betashe). As antigen for precipitin tests in diagnosing fascioliasis in cattle, Hoffman and Rivero used ether and alcohol Soxhlet extracts of dried liver flukes, dissolved in saline or Coca's solution. Kellaway's studies have shown that two antigenic substances occur in the liver fluke, which are active in the Dale guinea pig uterine strip test: (1) a true anaphylactogen, which occurs in the saline extract, and (2) a lipin capable of sensitizing an animal but not of affecting shock, which is present in the alcoholic extract.

(*b*) Cestoda. Among cestode infestations, hydatid disease is the only one in the diagnosis of which immunologic procedures have been widely used. Normal human hydatid fluid has by some been found to be the best antigen (Cantini, 1929a and b), while Goodale and Krischner phenolized the Berkefeld filtrate of the fluid. Dennis recently reported a method for preparing a stable antigen from the hydatid fluid: The antigen is precipitated from the fluid by trichloro-acetic acid, washed with distilled water, dissolved in weak alkali and reprecipitated, this time with acetic acid. The precipitate is dried, powdered and stored for use. When dissolved in 1,000 parts of alkaline physiologic solution of sodium chloride, the solution represents a tenfold concentration of hydatid antigen as compared with that in the natural fluid. The antigen serves well for precipitin, complement-fixation and skin tests. Zontschew reported that an alcoholic extract of hydatid fluid is nonspecific and therefore unsuitable for either complement fixation or skin tests for hydatid. Kellaway, Fairley and Williams studied the antigenicity of ultrafiltrates of hydatid fluid in pyroxylin membranes. Filtrates from fluid in membranes hard-

ened in 50 to 60 per cent alcohol contain no antigen detectable by shock, by complement fixation or by skin test and give no chemical reaction for protein. Filtrates from membranes hardened in 70 to 80 per cent alcohol give positive Spiegler and hemochromogen reactions, react well in anaphylactic tests and elicit skin reactions but act only poorly in complement fixation tests.

(*c*) Nematoda. The large amount of work on the diagnosis of trichinosis has been done chiefly with a Coca's solution extract of powdered dry larvae which have been obtained from infected muscle by digestion. Such an antigen has been found useful for precipitin tests (Bachman), complement fixation tests (Bachman and Menendez) and skin tests (Augustine and Theiler), although some investigators (Trawinski, 1934 and 1936d) have felt that a saline extract is preferable for intradermal use. Kaplan, working in this laboratory, prepares a trichinella antigen suitable for precipitin tests from larvae which have been obtained from heavily infected muscle by digestion, then dried over calcium chloride and powdered in a mortar. He adds the powder to Coca's solution in sufficient quantity to make a concentration of 1 per cent and permits this to extract at room temperature for seven days. The suspension is centrifugated at high speed, and the supernatant fluid is decanted and used for the tests. In making skin tests for filariasis, saline extracts of dried and powdered adults of Dirofilaria immitis proved satisfacory as antigen (Taliaferro and Hoffman; Fairley), although Fairley prefers an alcoholic extract for complement fixation tests. Gutierrez used a saline extract of dried, triturated adults of Onchocerca, freed from nodules by digestion, as antigen in complement fixation tests for onchocerciasis. An alcohol-acetone extract of Ancylostoma duodenale was used in complement fixation tests for ancylostomiasis by Arnaud in the Belgian Congo.

Results with the Precipitin Reaction.—(*a*) Trematoda. Relatively little has been done in the diagnosis of the trematodiases by the precipitin reaction. Taliaferro, Hoffman and Cook (1928a and b) have been able to identify human infestation with Schistosomum Mansoni by this method. In 28 cases in which the feces showed eggs, 77 tests gave 63 positive and 14 negative results. In 5 cases in which no schistosomiasis was present, 24 tests gave 1 positive and 23 negative results. There was a definite tendency for persons with positive evidence of syphilis to give a positive precipitin reaction even when they did not show schistosomiasis. The most satisfactory antigen was prepared by extracting the dried and powdered livers of heavily infected snails with ether or absolute alcohol or both and then with Coca's solution, the final clear supernatant fluid being adjusted to p_H 7.4.

Hoffman and Rivero, using a Soxhlet alcohol and ether extract of dried liver flukes dissolved in saline solution and in Coca's solution, found that all of 46 animals which showed evidence of infection at autopsy also showed infection by the precipitin test. However, in 39 of 101 other instances, positive serologic evidence was obtained in the absence of evidence of infection at autopsy.

(*b*) Cestoda. The occurrence of cysticerci in man can be recognized by the precipitin reaction. Rothfeld described 4 cases of human cerebral cysticercosis, in which Trawinski and Rothfeld were able to

make a diagnosis by the precipitin reaction. In 2 of these cases, the diagnosis was later confirmed, in one by excising the larvae from muscles just under the skin, and in the other by section of the brain. Trawinski (1936) was able by the precipitin test to identify an infection with Cysticercus cellulosae in pigs. Of 25 infected pigs, 22 were proved to harbor the parasites by the precipitin technic. The serums of all of 30 uninfected pigs were negative.

The precipitin test has not been found particularly satisfactory in diagnosing hydatid infection in man. Hoder stated that it was not specific and that it gave positive results in only about half of the cases which he had studied; Bonelli was unable to detect precipitin in the serum of cattle, sheep or pigs infected with hydatids.

(*c*) Nematoda. A number of papers have appeared on the use of the precipitin reaction in diagnosing trichinosis of man following Bachman's description of the method as useful in the case of rabbits. Hunter, as well as Bachman and others, applied the test with success for the diagnosis of human trichinosis. Augustine and Theiler believed the test to be always positive in human cases in which the fact of infection with this parasite had been established, although they obtained positive results also in patients treated for malaria with quinine. In diagnosing infestation of pigs they found the precipitin test highly specific. Theiler and Augustine considered the immunologic test particularly useful because of the ambiguity and occasionally complete absence of all other criteria. They, as well as Spink and Augustine, found the precipitin test positive in from four to five weeks after infection. Spink and Augustine noted that sporadic cases and cases in which trichinosis was chronic or in which it was mild were often detected only by the precipitin test or other immunologic procedures. Trawinski and Maternowska found the precipitin test specific, since antigens prepared from Ascaris and Echinococcus failed to react with the serums of infected animals. Trawinski suggested the precipitin test as the best means of diagnosing trichinosis in slaughtered animals. On the other hand, Heathman found the precipitin test of less value as a laboratory aid in diagnosing trichinosis than eosinophil counts together with biopsy of muscle and study of infected meat.

The precipitin test has not been used so widely in the diagnosis of any other nematode infection as it has in that of trichinosis. Stumberg (1930) was unable to detect precipitins for Ancylostoma canium in the serums of infected dogs up to the seventh week of infection.

Results with the Complement Fixation Reaction.—(*a*) Trematoda. Fairley (1933) established the fact that animals exposed to cercarias respond by the development of complement-fixing antibodies; 9 of 11 infected goats showed positive complement fixation. He showed further

that the test for the fixation antibody is useful in diagnosing human bilharzial infections, 24 of 26 patients having given positive fixation. Other investigators of the same problem (Andrews; Salam) have since reported similar results. For example, Andrews noted that fixation was obtained with the serums of 49 human beings and 5 dogs infected with Schistosomum japonicum, in the stools of which in all instances eggs were present. In 13 additional cases in which eggs were present in the stools, however, the test was negative. This suggests that a negative complement fixation test is not conclusive evidence that the patient is free from bilharzial infection.

Wagner obtained a positive diagnosis in 90 per cent of cases of infection of sheep with Fasciola hepatica by using the complement fixation test, supplemented by the skin test. He asserted, however, that infection is more certainly and more easily diagnosed by fecal examination than by immunologic procedures.

(*b*) Cestoda. Although some workers have been unable to detect specific antibodies in the serums of animals proved to have hydatid infection (Bonelli; Eristawi), the majority of workers have found that the infection initiates the formation of antibodies both in man (Cantini, 1929a; Cuff) and in animals (Goodale and Krischner). Fairley and Kellaway noted that the complement fixation test is especially valuable in diagnosing recurrent or residual cysts in patients who have been operated on for hydatid, persistent fixation of 6 minimal hemolytic doses of complement for nine months after operation indicating further infestation. Joyeux called attention to the wide discrepancy in the percentage of positive findings by different workers using complement fixation for the diagnosis of hydatid, stating that Lemaire reported but 17 per cent of the findings positive, whereas K. D. Fairley found 84 per cent positive. Hoder has since reported as high as 90 per cent positive results in cases of hydatid infection. Goodale and Krischner obtained positive fixation in the serums of 26 of 44 cows infected with hydatids, and false positive fixation in the serums of 10 of 62 noninfected animals.

Bonnall and co-workers reported their failure to detect specific complement-fixing bodies in the blood of a woman infected with a coenurus of Multiceps serialis, which was proved viable by feeding it to a dog, seven scolices being later recovered from the animal.

MacArthur reported that in 5 of 9 cases of epilepsy which he had proved to be caused by cysticercosis the condition was diagnosed correctly by N. H. Fairley by means of the complement fixation test.

Mueller and Chapman detected sparganosis of rhesus monkeys by the complement fixation test, using as antigen a saline extract of Diphyllobothrium Mansonoides. A positive reaction was obtained when as few as 2 spargana were present in the tissues. Ravetta reported that extracts of adult worms give complement deviation with the serums of dogs infected with Diphyllobothrium erinacei.

(*c*) Nematoda. Fairley (1931) detected complement-fixing antibodies in the blood of patients with filariasis and considered the test of some use in identifying the more active infections. He (1932) found that in cases of infection with Wuchereria Bancrofti and with Loa loa the reactions were strongly positive when microfilarias were in the blood but generally negative when elephantiasis developed. Fairley (1932)

employed as antigen an alcoholic extract of powdered Dirofilaria immitis. Others (Connal; Lloyd and Chandra) also employed the complement fixation test with success in filarial infection. Gutierrez and van Hoof demonstrated the complement-fixing antibody in onchocerciasis, the latter worker finding it in the serum, cerebrospinal fluid and synovial and edematous fluids.

Arnaud found that human carriers of hookworms usually give positive fixation with an alcohol-acetone extract of the worms, although Stumberg (1930) noted that dogs experimentally infected with Ancylostoma canium failed to show the complement-fixing antibody up to seven weeks after infestation.

Blackie (1931) obtained complement fixation during the first five weeks of infection of rabbits with Ascaris megalocephala. On the fifteenth day after exposure of the animals, their serum fixed 18 minimal hemolytic doses of complement per cubic centimeter.

Results with Intradermal Tests.—(*a*) Trematoda. Fairley and Williams showed that skin tests could be used to identify infections with Schistosomum haematobium. Seven of 8 patients who were known to be infected with Schistosomum haematobium gave immediate reactions in skin tests with an antigen prepared from dried and powdered livers of Planorbis exustus infected with Schistosomum spindale. Three of these immediate reactors also gave a delayed reaction. Of 47 control persons, 44 failed entirely to give a reaction and the remaining 3 gave only the immediate type of response. Vogel confirmed the essentials of the Fairley and Williams test, finding that it can be used as well on Negro subjects. The Taliaferros also studied the problem, using an antigen prepared from livers of Planorbis guadelupensis infected with Schistosomum mansoni. They found that most infected persons gave immediate reactions but that marked delayed reactions were rare. The Taliaferros were able also to perform the Prausnitz-Küstner test by injecting the serum of an infected person into the skin of a normal person and inoculating the same site subsequently with the specific antigen. Later Khalil and Hassan reported that of 136 persons passing the ova of schistosomes, all but 4 gave immediate reactions and all but 2 gave delayed reactions with an antigen prepared from adults of Schistosomum bovis. Kan (1936b), however, found that only 60.4 per cent of those giving positive skin reactions for Schistosomum japonicum also were proved by examination of stools to have the eggs in their feces. This worker feels nonetheless that the skin test is useful in field work, since only 1 per cent of persons proved to have the infection failed in his hands to manifest positive skin reactions on intradermal injection of the antigen prepared from Schistosomum japonicum (Kan, 1936a). Generally the skin reaction persisted long after apparent cure by chemotherapeusis (Fairley and Williams; Manson-Bahr; Khalil and Hassan).

The results of skin tests to diagnose fascioliasis are less satisfactory. Curasson found skin tests of only little value for identifying liver fluke and amphistome infections in sheep or cattle. Wagner, as well as Sievers and Oyarzun, found such tests useful, but Wagner considered direct search of the feces for eggs of the parasite simpler and more certain for diagnosis. The skin test was found positive in the case of a native of French Somaliland who was known to be infected with Fasciola hepatica as well as with Trichocephalus (Heckenroth and Guilliny).

(*b*) Cestoda. Intradermal tests, such as that first described by Casoni, have been used extensively in the study and diagnosis of hydatid infection (von Bassewitz; Berthier; Cuff; Fairley and Kellaway; Goodale and Krischner; Mollow; Zontschew). Usually a higher percentage of correctly positive results has been obtained by the skin test than by any other biologic method. Hoder, for example, found that he could identify 100 per cent of the cases by the skin test, 90 per cent by the complement fixation test and 50 per cent by the precipitin test. Kellaway and Fairley considered that the skin test identified at least 75 per cent of hydatid infections in patients before operation. Goodale and Krischner noted that of 44 cows infected with hydatids, 38 gave positive skin reactions and only 26 positive complement fixation reactions. The same workers reported that of 62 uninfected cows, 11 gave false positive skin reactions and 10, false positive complement fixation reactions. Wild animals in captivity (antelope, lama and others) known to harbor the parasite gave only 68.5 per cent positive results in the hands of Urbain. Longo pointed out that the delayed reaction is a better indication of infection than the immediate reaction, since the latter is given by many uninfected persons. In his work, 23 of 100 noncarriers gave immediate reactions, but none gave delayed reactions, following the injection of 0.5 cc. of hydatid fluid. Kellaway and Fairley considered that the absence of an immediate response correctly shows freedom from infection in 90 per cent of the persons tested. It has been generally found that a positive skin reaction with hydatid fluid occurs in any infection with Taenia (Morenas, 1933; Nuñez and Lopez, 1933; Nuñez), including human infection with Taenia saginata (Morenas, 1929).

Most workers have reported that the positive skin reaction persists often for years after the hydatid cyst has been removed surgically (von Bassewitz; Kellaway and Fairley), although Morenas (1929) found the test negative two months after removal of a cyst from the liver. It seems possible that the long-continuing skin reaction indicates either reinfection, infection with daughter cysts or incomplete removal of all the cysts by operation.

As would be expected, intestinal infection of dogs with adult hydatids does not induce a response demonstrable by skin tests with hydatid fluid (Turner and co-workers, 1935b). Morenas (1933) was able in several cases to diagnose cerebral

cysticercosis by means of a skin test with the fluid of any Taenia cysticercus, coenurus or hydatid, although Bonnall and co-workers failed to diagnose an infection of a patient with the coenurus of Multiceps serialis.

(*c*) Nematoda. Among infections with nematodes which can be diagnosed by means of the skin tests, trichinosis stands out conspicuously. Following Bachman's original work on trichinosis in rabbits, as well as on that in man, many investigators have directed their attention to the problem. One worker, Musger, found the test positive as early as the fourth day after infection, and most experimenters have found it positive by the third week (McCoy, Miller and Friedlander; Theiler and Augustine). The persistence of the positive reaction for from four to nine years after recovery has been observed (Theiler, Augustine and Spink). Some have found the skin test less satisfactory than the older procedures—biopsy of muscle and eosinophil counts (Heathman; Kilduffe, 1933 and 1936; Kaljus)—but nearly all have agreed that the skin reaction has some merit in diagnosis, and many have considered the skin test the method of choice, particularly when it is supplemented by the precipitin test (Augustine and Theiler; Drake, Hawkes and Warren; Friedlander; Maternowska, 1933; Rodenwaldt, 1934a). Nevertheless, McCoy, Miller and Friedlander, who found that 90 per cent of those ill with trichinosis gave positive skin reactions, considered that a negative test is even more conclusive, being essentially absolute evidence of freedom from infection. They believed that occasionally a positive skin reaction to antigen prepared from Trichinella may be induced by an infection with the closely related nematode, Trichuris trichiura, or that it may be obtained because of sensitization remaining from an earlier infection with Trichinella from which the patient has entirely recovered at the time of testing. In the latter case, the diagnosis, although correct, is misleading, since it may cause the physician to relent from further search for the actual cause of the symptoms from which the patient is suffering. Otto (also Otto and Janney) reported the successful application of the skin test for the diagnosis of trichinosis in a family in Maryland, whose afflictions had previously been variously diagnosed as typhoid fever, acute alcoholism, influenza complicated by chronic bronchitis, mild encephalitis, influenza, mumps and rheumatism.

The skin reaction generally sets in at from four to eight hours after the test injection and lasts about fifty hours (Trawinski, 1936d). In man, two phases—an immediate and a delayed response—can be differentiated. In the first, or edematous, stage a papule is produced, which reaches its maximal size in about nine hours. In the second, or infiltrative, stage the maximum is reached in from twenty-four to forty-eight hours. With old infections the second stage is revealed more prominently than the first stage. The two stages coincide in animals and usually can not be differentiated (Maternowska, 1933a and b). Spink found that the immediate type of reaction is generally not elicited until the second week of infection, the reaction in the first few days being of the tuberculin type. (See also Augustine.)

The skin test has also been applied quite extensively in the detection of filariasis. Fairley (1932), as well as Rodhain and Dubois (1932), using Dirofilaria immitis as antigen, obtained positive skin reactions in infections with Wuchereria Bancrofti, Loa loa and Onchocerca volvulus. Fairley (1932) considered that the skin test taps residual intracellular

antibody and can be expected to persist long after circulating antibody as well as the parasites themselves have disappeared. Connal used Fairley's test with success in diagnosing Calabar swelling, and d'Hooghe, although questioning their reliability for diagnosis (d'Hooghe, 1935), obtained positive skin reactions in onchocerciasis and loiasis, the antigen he employed being Onchocerca volvulus (d'Hooghe, 1934). The results of all of these workers, as well as those of other workers (Rodenwaldt, 1934b), called attention to the group nature of the skin reactions in patients with filariasis. In a patient infected with Loa loa, Chandler and associates were able not only to elicit a skin response on injecting an antigen prepared from Dirofilaria immitis but to produce typical Calabar swellings, thus providing evidence for the allergic nature of these swellings, as Fulleborn first suggested. Sayers accorded significance to negative tests, concluding that, since tests with an antigen prepared from Dirofilaria immitis were negative in a series of cases of tropical myositis, this disease could not be regarded as a complication of filariasis. Ramsay, using a guinea worm antigen, obtained positive skin reactions in 35 of 41 patients who were infected with guinea worms.

In general, workers have not found the skin test satisfactory for the diagnosis of ancylostomiasis (Arnaud; Pirie, Retief and Ferguson). Bachman and Rodriguez-Molina (1932a and b) considered that skin sensitiveness persists indefinitely after the elimination of the parasite. Stumberg and Rodriguez-Molina also were unable to note any relationship between the positive skin reaction and hookworm infection, except for some correlation between the duration of the skin test wheal and active disease. In general, however, Stumberg and Rodriguez-Molina considered the result of the skin test too variable for reliable diagnosis. In contrast to all of the workers just cited Vattuone obtained intense skin responses in patients with severe ancylostomiasis as compared with those in essentially healthy carriers, although even the latter gave marked reactions. Some patients who were so lightly infected as to be thought free from infection on fecal examination gave positive skin reactions. Kitamura showed that rabbits previously given injections of Ringer's solution containing the metabolic products of the larvae of hookworms give more intense reactions to skin penetration by ancylostomes in the infective stage than rabbits not so treated. An essentially similar observation on Strongyloides stercoralis was reported by Sato, who noted that marked skin reactions appear at the sites of penetration of the infective larvae in animals previously artificially immunized to this species.

Similarly, no correlation is found between ascariasis and skin sensitivity to ascaris antigen (Bachman and Rodriguez-Molina, 1932a and b; Diehl and Schwoerer; Hegglin; Hofmann; Jadassohn; Khaw). Jones and Kingscote, although noting that a positive skin reaction to ascaris antigen did not always indicate a prior infection, believed a period of exposure to the parasite a necessary precursor of sensitivity. Konus and Gakoubovitch obtained 68.5 per cent accuracy in the diagnosis of ascariasis in children by skin tests, and Schönfeld considered that a strongly positive immediate skin reaction should lead one to suspect the presence of worms even when ova are lacking from the stools.

Wright and Bozicevich obtained promising results with dermal and intradermal tests for diagnosing pinworm infection, using as antigen an extract of larval and adult Oxyuris vermicularis.

Results with Tests for Antigen.—Practically nothing has been done toward developing tests for the antigen of helminths in patients or animals. Sievers was unable to detect a specific antigen in the serum of carriers of Diphyllobothrium latum, although Stumberg (1933) demonstrated, by an anaphylactic reaction, a protein of Haemonchus in the blood of an infected sheep. The Stumberg test consists in producing a rise in the relative percentage as well as in the absolute number of eosinophils by injecting the blood of a sheep infected with Haemonchus into a guinea pig which was previously sensitized by an extract of Haemonchus contortus. It seems possible that other acute helminthic diseases can also be diagnosed by this means, especially in their early stages before antibody can be formed against the invader. Indeed, attention has been called (in an editorial in the *Lancet*, 1932, p. 1167) to the fact that antibody appears only after the patient is well along toward recovery, when the diagnosis is relatively unimportant, except to confirm the clinician's diagnosis.

Specificity of Immunologic Tests in Diagnosis.—(*a*) Trematoda. A great similarity in the antigenic constitution of all mammalian schistosomes is shown by the fact that the cercarias of any mammalian schistosome may be used as the antigen to detect antibody called forth by an infection with any mammalian schistosome. Therefore, the reaction is clearly a reaction to the group, and infection with any of the human schistosomes produces antibodies reactive with antigen prepared from Cercaria spindalis (Fairley and Jasudasan). However, the nonspecific character of the reaction is not without definite limits. Fairley (1926 and 1933) concluded that a positive complement fixation test in which as antigen an alcoholic extract of infected snail livers (Planorbis exustus infected with Schistosoma spindalis) was used almost invariably means infection with some mammalian schistosome or, as a notable exception, with the liver fluke, Fasciola hepatica. Quite in agreement with the last observation, the antigen of Fasciola gigantica has been used successfully in skin tests for human schistosomiasis (Hassan and Betashe). The liver fluke antigen also is found to induce a positive skin reaction in infections with Dicrocoelium (Wagner).

(*b*) Cestoda. It has also been rather well established that infection with, or parenteral administration of, the antigens of one species of Taenia induces antibodies which will react against the proteins of different species of Taenia (Nuñez; Nuñez and Lopez, 1934). Morenas (1932), after showing that carriers of Taenia saginata give the Casoni reaction with hydatid fluid, has found the converse to be true—that patients infected with hydatids react to the fluid obtained from Cysticercus tenuicollis of sheep. The Fairleys, K. D. and N. H., and Williams considered that not only other species of Taenia but also nematodes cause positive skin reactions, both immediate and delayed, with hydatid fluid. They found that such conditions as jaundice, asthma, urticaria and pruritus would cause pseudo-reactions to hydatid fluid. Pickhardt reported that sometimes hydatid infection leads to a positive Wasser-

mann reaction, which persists despite intensive specific postoperative treatment.

(*c*) Nematoda. A skin reaction to trichinella protein generally indicates infection with Trichinella spiralis, although McCoy, Miller and Friedlander have shown that patients infected with Trichuris trichiura also give positive skin reactions to the trichinella antigen. Maternowska (1933b) found patients with trichinosis to be unresponsive to ascaris proteins.

Rodhain and Dubois (1931) reported that European patients in the Belgian Congo infected with Onchocerca volvulus react equally well to skin tests with antigens from Onchocerca volvulus, Loa loa and Ascaris. Fairley (1931 and 1932) found that antigens of Dirofilaria immitis serve to test for loiasis, onchocerciasis and filariasis except when elephantiasis develops.

V. OTHER IMMUNOLOGIC PHENOMENA OF SIGNIFICANCE IN HELMINTHOLOGY

In the following paragraphs various subjects which have immunologic significance are discussed. Unfortunately, in some cases the volume of work reported is small, and the actual value of the observations is obscure.

TOXINS OF PARASITES

A number of helminths are believed to produce a toxic substance to which the body potentially may form an antitoxin (Fülleborn and Kikuth). Elsbach stated that the adult filarial worm elaborates a toxin which affects the lymphatic endothelium but not the blood endothelium, the latter tissue being protected by the function of the liver in detoxifying the substance. Grace expressed the belief that the condition of elephantiasis, also, represents the effects of a toxin, although he considered that the toxin comes from Streptococcus haemolyticus and that Wuchereria Bancrofti plays no role in elephantiasis. Van Hoof reported that a toxin is present in onchocerciasis. The symptoms of gastritis in sheep infected with Haemonchus contortus are possibly due to a toxin which this parasite elaborates (Taylor). The toxic symptoms in trichinosis may be caused by absorption of large amounts of muscle destroyed and rendered toxic by the invading trichinas rather than by a toxin coming from the parasite itself (Stoll, 1929). Fülleborn reported that Kikuth observed a heat-stable hemolysin in high concentration in Ringer's solution in which Ancylostoma canium were kept for several days. As yet, however, it has not been proved that a true toxin (in an immunologic sense) is produced by any helminth.

Sensitivity to Ascaris.—The ascarids are often believed to form toxins. One author reported 2 cases of human ascariasis in which febrile symptoms were observed which he considered to result from intoxication, although he was undecided whether live or dead worms produced the toxic substance (Pruis). Koidzumi, who studied ascaris toxicity extensively, found the filtrate of muscle cells more toxic than the body fluid. Animals given intravenous or subcutaneous injections of ascaris material showed characteristically a histamine type of shock.

Possibly the reason that the majority of experiments designed to show a correlation between skin sensitivity to ascaris material and infection have failed (Hegglin; Khaw, and others) is that the ascaris substance is natively toxic. Mu was able to carry out the Shwartzman reaction in rabbits into which he had injected an extract of Ascaris lumbricoides first intradermally and after twenty-four hours intravenously. The Shwartzman reaction appears, however, in the light of recent work, not to involve an antibody or to require the hypersensitive state. Fishback demonstrated the hemotoxic action of saline, methyl alcohol and acetone-insoluble, alcohol-soluble extracts of Ascaris lumbricoides or Ascaris suilla on human red cells. Blackie (1930) showed that mice, rabbits and guinea pigs infected with the larvae of Ascaris megalocephala manifested renal injury despite the fact that the larvae do not migrate through the kidney. Yet others have considered the positive skin test an indication of the hypersensitive state (Jadassohn), if not through prior infection, at least through exposure to ascaris materials (Jones and Kingscote). Jadassohn found that 80 per cent of children between 2 and 4 years of age gave positive skin reactions, although children under 1 year never, and older persons seldom, gave them. Fellenberg, supporting the sensitization theory, reported the case of a mother manifesting marked urticaria on each of several occasions when her son suffered with ascariasis, her condition improving when the boy lost his worms. Yet some support for the opposite point of view comes from Dubelsky and Golubewa's experiment of submitting 10 normal guinea pigs to injection of body fluid from Ascaris megalocephala. Only 3 of the pigs failed to show some symptoms, and 2 died of the injection. Emery and Herrick studied the circulatory and respiratory changes produced by injections in etherized dogs and in cats anesthetized with a compound of chloral hydrate and dextrose. The blood pressure was strongly depressed, and the respiration was deeper, although the heart rate and the respiration rate were unaltered. These workers found that the extract irritated the skin and the respiratory tract of man, the specific effects resembling those of histamine.

ADHESION PHENOMENON

The adhesion reaction has had frequent use in the study of spirochetal and trypanosomal infections but has been used but little in that of helminthiases. Positive reactions have been obtained under certain circumstances in filariasis. In these, the microfilarias become sluggish or lose activity and are coated with adherent leukocytes. Red cells do not adhere. Pandit and co-workers obtained adhesion with the blood of 25 of 32 persons with elephantiasis in whom nocturnal blood invasion by microfilarias was occurring. In the blood of 10 patients with blood invasion but without lesions, the test gave negative results. Only 3 of 13 healthy persons showed adhesion. The test was found specific in respect to the species of microfilarias. Pandit and collaborators considered that the usual absence of microfilarias from the blood in cases of elephantiasis is explained by the adhesion phenomenon.

AGGLUTINATION OF GRANULES IN THE VAS DEFERENS OF ASCARIS

Momma described an agglutinative action of normal serum from man, the dog, the cat and the rabbit on granules obtained from the vas deferens of Ascaris. He found that the granules absorb the agglutinative agent from the serum and that a serum absorbed with pig or human ascaris granules will not thereafter agglutinate those of either the same or the alternate Ascaris, but will produce a reaction with

granules from Toxocara mystax, Toxocara canis or Toxocara equorum. Momma considered this as serologic evidence of the identity of Ascaris lumbricoides and Ascaris suilla.

SCHISTOSOMAL CERCARIAL DERMATITIS

A considerable percentage of persons manifest dermatitis after exposure to the cercarias of some species of schistosomes (Cort, 1928). Usually merely a prickling sensation is experienced at the time the cercaria effects penetration, although on the next day a distinct papule appears, which gradually attains greater size and severity during the ensuing three or four days. Thereafter, the reaction generally subsides, but traces of the lesion are often discernible after from four to seven weeks (Taylor and Baylis). Patients vary widely in their response to the cercarias; in many no dermatitis develops. It is possible that a person must be sensitized through prior exposure before he can manifest the dermatitis, although this has not been established as yet. Some evidence has been offered that repeated attacks by the cercarias of animal schistosomes sensitize the subject so that he gives a reaction when tested intradermally with antigens of human schistosomes (Cort, 1936c). An attack in which the skin reaction develops appears to confer no immunity against a second attack.

Cort's studies of schistosomal dermatitis were made with the cercarias of schistosomes of the Great Lakes area, five distinct species being known to cause the disease (Cort, 1936d). These forms develop normally in rodents and other wild forms of the region and never in man. Opinion is divided as to whether the cercarias of schistosomes are able to elicit dermatitis on penetrating the skin of their normal hosts. One author, Watarai, reported that the cercarias of Schistosomum japonicum cause no dermatitis in man but that a very few or even one single cercaria of this species will cause dermatitis in the domestic fowl. He concluded, therefore, that kabure, an itching dermatitis known among Oriental peoples since the ancients, is caused by larvae of some heterogeneous trematode, cestode or nematode and not by the larvae of Schistosomum japonicum. Miyagawa, many years earlier, reported that he and an assistant had contracted kabure after immersing their arms in the water of rice fields without schistosomiasis developing, and stated that the disease occurred in regions where schistosomiasis was not known. Conversely, Faust and Meleney noted no skin eruptions among persons within the schistosome-infested areas. Faust, Jones and Hoffman were unable to find evidence of dermatitis in Puerto Ricans after contact with water containing cercarias of Schistosomum Mansoni.

On the other hand, Vogel noted slight dermatitis in the arm of a white man exposed to cercarias of Schistosomum Mansoni, although 2 Negroes failed to show such a reaction. Barlow presented evidence from his work in Egypt that cercarias of the human schistosomes of

Egypt cause the dermatitis observed in many of those whose occupation requires work for many hours in water. He noted that the itching was most severe between 9 a. m. and 5 p. m. and that it did not occur at night. Infected snails of the region were found to shed cercarias of the human schistosomes from 9 a. m. till noon. They were found, furthermore, to harbor no schistosomes besides the human ones. Barlow produced the dermatitis experimentally in 43 persons, using as the inciting agent cercarias of both Schistosomum haematobium and Schistosomum Mansoni.

CERCARICIDAL ACTION OF NORMAL SERUM

Although it has long been known that the normal blood serum of many animals possesses bactericidal power and that the normal serum of man and a few other Primates has the additional capacity to lyse some species of trypanosomes and to neutralize certain filtrable viruses, no helminthicidal property of normal serum was known until recently. Culbertson and Talbot tested the normal serums of various animals, representing all the classes of vertebrates, for antagonistic action against different trematodes in the cercarial stage and in many instances obtained positive results, although exceptionally, particularly with the serum of cats, no such effect was seen. Likewise the cercarias of certain species of trematodes were able to survive quite as well in serum as in the control fluids in which they were kept. The substance in the serum responsible for the cercaricidal effect is highly labile, being readily destroyed by heat or desiccation and quickly lost in storage. Agencies which destroy alexin also destroy at the same time the cercaricidal power of the serum. It seems unlikely that the cercaricidal action of serum arises necessarily following specific infection with a given trematode or with related forms, for the blood of laboratory-bred, trematode-free rats manifests this action (Culbertson). It is possible that eventually the test for cercaricidal action of serum may aid in determining potential hosts of cercarias in the life histories of trematodes. So far as I am aware, no similar action against groups of helminths other than trematodes is known.

Nigrelli has since described a similar action of the mucus from certain species of fish on monogenetic trematodes. He found that mucus from the naturally resistant dogfish rendered the parasites moribund in three hours and killed nearly all of those exposed in five hours. When the parasites were exposed to mucus from the highly susceptible pompano, they remained alive usually for from eighteen to twenty-four hours, and if washed several times, for three days. He concluded that the action of the mucus played some part in the protection of the fish against these parasites.

IMMUNITY REACTIONS AGAINST TREMATODES IN SNAILS

An interesting new line of evidence of immunity against helminths was opened by Winfield (1932), who noted that when specimens of two varieties of the snail Lymnaea stagnalis are infested with the sporocysts of the strigeid Cotylurus flabelliformis they become highly resistant to penetration by cercarias of this species, which normally penetrate and develop in uninfected snails of the two varieties mentioned. Autopsies made on snails which were uninfected and snails from which cercarias had been escaping for eleven days, after both groups were exposed to cercarias, showed an average of 1,912 metacercarias and 4.8 metacercarias, respectively, per snail. It was believed that this immunity played a part in the survival of snails which harbored this trematode, since it protected them from being immediately attacked and overwhelmed by the great numbers of cercarias which escaped from them.

Nolf and Cort investigated the specificity of this immunity. In some cases, no nonspecific immunity as the result of infection could be demonstrated. For example, when snails (Stagnicola emarginata-angulata) were exposed to cercarias of the same genus but of a different species (Cotylurus communis) of trematode from that with which they were infected (Cotylurus flabelliformis) no evidence of immunity was elicited, approximately as many metacercarias forming as in uninfected control snails. On the other hand, two varieties of Lymnaea stagnalis infected with schistosomes (Schistosomatium Douthitti) in the larval stage gave evidence of a partial nonspecific immunity to penetration by cercarias of Cotylurus flabelliformis. In this case significantly larger numbers of metacercarias formed in the control snails, and these metacercarias were much larger than those found in the infected snails.

Cort, McMullen and Brackett studied carefully natural double infection of snails. They collected 2,526 specimens of Stagnicola emarginata-angulata from three localities on Douglas Lake, Mich. In 1,809 instances in which infection was demonstrated, the snails shed fifteen species of cercarias. Of these infected snails, 172 had double infections with fifteen different combinations. With five combinations in which each of the two forms was a strigeid, as well as with a combination of a strigeid and a schistosome, the actual number of snails found agreed with expectations under chance infection, suggesting that no immune responses were demonstrable. However, some evidence of immunity was adduced through the failure of occurrence of double infections with the cercarias of Diplostomum flexicaudum, which had an incidence of 40 per cent in all of those collected and with three other species of cercarias. Double infection was not found to occur at all in connection with an echinostome or a xiphidiocercaria (Cotylurus polyadena), although the chance expectancies were 20 and 41, respectively, and it was found only 5 times in connection with the commonest xiphidiocercaria when this combination should have occurred 161 times, if governed only by chance.

MISCELLANEOUS NONSPECIFIC REACTIONS

Aminopyrine Test.—Van Slype (1932) investigated whether the aminopyrine reaction in serum (due to absorption of shed blood) is upset by parasites which draw blood but was unable to reach a definite conclusion. He found that the degree of reaction did not run parallel with the number of eggs determined by the Stoll technic and that the elimination of hookworms did not always make a positive reaction nega-

tive. Among 26 patients with ascariasis, 8 gave strongly positive and 6 feeble reactions, while 10 gave no reaction. However, all had originally a multiple infection with other helminths, the state of single infection being reached by treatment (van Slype, 1933).

Formaldehyde Test.—Popesco found the formaldehyde test useless for diagnosing infection with Dirofilaria immitis in dogs. Of 44 infected dogs tested, 10 gave positive and 8 doubtfully positive reactions, whereas 26 gave negative reactions. In 34 dogs showing no microfilarias, the reactions were positive in 6, doubtfully positive in 3 and negative in 25. The reaction was previously found anomalous in Taenia infections.

SUMMARY

It is the purpose of the entire paper to summarize the literature on immunity to the helminths, and an appended summary is hardly necessary. Probably this paper, nevertheless, could close most satisfactorily with a brief résumé of recent work which in my estimation has been particularly significant and which has been done on a sufficiently large scale to establish fairly well the conclusions the investigators drew.

While as yet little headway has been made toward explaining the natural resistance or susceptibility of certain species of animals to helminths, the answer seems in certain cases to lie rather clearly in the action of the digestive juices. For example, Berberian has found that the intestinal juice of several species of animals which cannot be infected with hydatids in the adult stage has the capacity to digest the larval cestode used for infection, whereas that of the dog, which is the natural host for the adult worm, cannot digest the larva.

Evidence has been offered for and against Sandground's hypothesis that the resistance which comes with age is shown by hosts only or chiefly against abnormal parasites. Sarles (1929a) demonstrated such resistance in both dogs and cats against the dog hookworm Ancylostoma duodenale but Foster (1936b) held that resistance of this type is simply a reflection of the natural curve of the hemoglobin level of a given host with age pointing out that agencies causing anemia in the older hosts reduce or overcome their natural resistance.

The acquisition of immunity by infection requires that the host suffer injury so that it is caused to respond to the presence of the invader. Infection with helminths that cause little or no injury, therefore, leads to comparatively little immunity. For example, Miller (1932g) was unable to demonstrate in cats evidence of immunity against the adult cestode Taenia crassicollis, which dwells in the lumen of the cat's intestine without injuring the host. The same worker illustrated the relatively marked immune response against somatic helminths in his studies on the larval Taenia crassicollis (Cysticercus fasciolaris) in rats. The presence of one or more relatively large cysts of this cestode in the

liver of the rat wholly prevents subsequent infection of the animal when oncospheres of the parasite are fed to it (Miller, 1930).

McCoy (1931c) indicated, however, that an infected animal responds to Trichinella spiralis whether this is in the intestinal or in the somatic stage. Rats which have suffered an earlier infection with this parasite show fewer adults developing in the intestine from a standard feeding of infective larvae than do controls, and a proportionately smaller amount of muscle invasion by the embryos produced from these adults. In this case, the immunity seems directed perhaps chiefly against the intestinal parasite.

Some advance has been made in immunization of animals against helminths by means of killed antigens. Miller (1930) immunized rats against the cysticerci of Taenia crassicollis by injecting suspensions of dried, powdered worm material intraperitoneally at from two to three day intervals for five weeks. Sheep have been immunized, although incompletely, against hydatids by repeated preliminary injections of the fluid from the hydatid cyst (Turner, Dennis and Berberian, 1937), and rats have been protected against Trichinella spiralis by intraperitoneal administration of live larvae as well as by that of heat-killed larvae (McCoy, 1935).

Passive transfer of immunity in serum was effected by Miller (1932 and 1934), the serum of an infected rat protecting a normal rat against cysticerci of Taenia crassicollis. Sarles and Taliaferro accomplished the same result with the serum of rats infected with Nippostrongylus muris. Trawinski (1935) and later Culbertson and Kaplan presented limited evidence that passive transfer of immunity is possible in experimental trichiniasis.

Miller (1932d and 1935k) showed that mother rats naturally transmit to their offspring immune substances against the cysts of Taenia crassicollis, although the route of transfer of the substance has yet to be demonstrated.

Among agencies which affect the resistance of animals to helminths, the diet has been studied more than any other. Foster and Cort (1931 and 1932) showed that adult dogs on a poor diet become susceptible to infestation with the hookworm Ancylostoma canium, although the essential deficiency of the diet responsible for this enhancement in susceptibility is still obscure. A diet deficient in vitamin A renders rats less resistant to Trichinella spiralis (McCoy, 1934) as well as to Nippostrongylus muris (Spindler, 1933). A deficiency in calcium renders chicks more susceptible to infection with Syngamus trachealis (Clapham, 1934d). Repeated hemorrhage leads to greater susceptibility of dogs and cats and probably of man to infection with hookworms (Foster, 1936b; Cort, 1932) and of chicks to infection with Ascaridia lineata (Porter and Ackert).

Not only do fewer helminths develop in specifically immunized animals, as previously described, but those which develop are often smaller, as found by Miller (1930) with cysts of Taenia crassicollis in rats. Furthermore, in an immune host they may fail to reach maturity, although as reported by Chandler (1932a), they may resume growth if transferred to a normal animal. It has been suggested that something of the character of an antienzyme is responsible for the inhibition of growth in the resistant animal (Chandler, 1936g; Ackert, Porter and Beach).

Perhaps the most convincing evidence that antibodies are responsible for acquired immunity against helminths was supplied by Sarles and Taliaferro, who demonstrated a precipitate about and within the bodies of larval Nippostrongylus muris in the skin and lungs of immune rats. Responses of the Arthus type, which perhaps always indicate the action of an antibody, have been observed by Kerr (1936) in immune mice exposed to larvae of Ancylostoma and by Turner, Dennis and Berberian (1937) in immune sheep exposed to hydatids. An antibody against helminths has been found in serum, in pleural, peritoneal and pericardial fluids (Fairley and Jasudasan) and in synovial, cerebrospinal and edematous fluids (van Hoof). In infections of considerable severity, the antibodies generally reach their maximum by the fourth week, as indicated by Fairley and Jasudasan in trematode infections of goats and by Bachman and Menendez in rabbit trichiniasis.

The same kind of cells are involved in immune responses to helminths as in such reactions to other infecting agents, the macrophages being of greatest importance (Taliaferro, 1934; Kerr, 1935b and 1936; Blackie, 1930).

The application of immunologic phenomena for the classification of helminths has not advanced significantly as yet, although Fairley (1931 and 1932) and Eisenbrandt have made beginnings in this direction. On the diagnosis of helminthic infection a large literature has been contributed, which shows that immunologic tests are reasonably effective for diagnosis, especially for establishing the presence of somatic parasites (Schulz and Shikhobalova). The precipitin reaction has proved a valuable aid in identifying human schistosomiasis (Taliaferro, Hoffman and Cook, 1928a and b), cattle fascioliasis (Hoffman and Rivero), human cysticercosis (Trawinski and Rothfeld) and trichiniasis (Bachman; Augustine and Theiler; Trawinski and Maternowska). The complement fixation test is helpful in establishing the diagnosis of schistosomiasis (Fairley, 1933), sheep fascioliasis (Wagner), recurrent or residual hydatid infection (Fairley and Kellaway and filariasis (Fairley, 1931). Skin tests have been extensively tried and in some helminthic infections have proved valuable, particularly in schistosomiasis (Fairley and Williams; the Taliaferros), hydatidosis (Fairley and

Kellaway; Goodale and Krischner), trichiniasis (McCoy, Miller and Friedlander; Theiler, Augustine and Spink) and filariasis (Fairley, 1932).

A number of other problems of immunologic significance have held the attention of some investigators. The character of the schistosomal dermatitis first described by Cort (1928) has been studied, some investigators inclining to the point of view that dermatitis follows only when the invading schistosomal cercaria is of a species unable to develop further in man (Watarai). Winfield (1932) demonstrated immunity in the molluscan host against the cercarias which it sheds. The normal serum of a number of species of vertebrates has been shown to be cercaricidal (Culbertson).

It is apparent from what has been given here that the subject of immunity against helminths has been attacked by a large number of workers and that their contributions have covered many aspects of the whole field. Although there are still some who hesitate to agree that immunity to any helminth has been experimentally established (Schmid), the great majority of workers are more favorably inclined and tend to assume the point of view that the mechanism of immunity against helminths is similar to that against other infectious agents. The manner in which the state of immunity against helminths is attained, the humoral and cellular responses of the immune host against these parasites, and the effects of the defense agencies of the host on the parasites are so nearly identical with the immune phenomena associated with other types of infectious agents that one must either accept the fact that immunity is developed against the helminths or believe that no immunity is manifested against any agent of disease.

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Notes and News

Society News.—The Boston Pathological Society, which was organized in 1929, has changed its name to the New England Pathological Society. Its members are spread over the New England states. F. Parker Jr. is the president and F. B. Hazard is the secretary-treasurer. Regular meetings are held on the third Thursdays of October, November, January, February, April and May.

The American Public Health Association will hold its sixty-seventh annual meeting in Kansas City, Mo., Oct. 25-28, 1938.

Ernest Witebsky has been elected president and Samuel Sanes secretary of the Buffalo Pathological Society.

The third International Congress for Microbiology will be held in New York City, Sept. 2 to 9, 1939, under the presidency of T. M. Rivers. The congress will be composed of nine sections. The general secretary is M. H. Dawson, College of Physicians and Surgeons, Columbia University.

According to *Science,* the fifth International Congress for Experimental Cytology will be held in Zurich next August 7 to 12. There will be seven scientific sessions devoted to the following subjects: epithelium in cultures and in the organism; the structure of chromosomes; the mechanism of mitosis; cancer cells and normal cells; experimental cytology and the study of viruses; the ultrastructure of protoplasm and of its products; the chemistry of the cell. The congress will not be divided into sections. Papers should be submitted with summaries not exceeding 200 words before April 15 to Prof. W. von Möllendorff, 9 Plattenstrasse, Zurich. Copies of such papers will be distributed to the members of the congress before the meetings.

The sixteenth International Physiological Congress and the International Veterinarian Congress will be held in Zurich in the middle of August 1938.

Patterson Institute for Cancer Research.—Northwestern University, Chicago, has organized for treatment and research the Patterson Institute for Cancer Research by means of a donation of about $500,000.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Renal Insufficiency from Blood Transfusion. E. L. DeGowin, R. F. Osterhagen and M. Andersch, Arch. Int. Med. **59**:432, 1937.

Intravenous injections of large amounts of dog hemoglobin into dogs seems to be innocuous if their urine is alkaline. Under such conditions each of four dogs received thirty-eight transfusions without harm. In one dog retention of nitrogen developed on three occasions when a transfusion was given while the urine was acid. When the urine is acid, transfusions of hemoglobin sooner or later produce renal insufficiency. Seven dogs died in coma from four to ten days after transfusion, with the urea nitrogen of the blood ranging between 120 and 362.6 mg. per hundred cubic centimeters and the creatinine between 4.6 and 15 mg. In one dog killed when recovering the urea nitrogen content of the blood was 95 mg. Only one dog survived seven transfusions when on an acid diet. This syndrome clinically and chemically closely simulates that which develops in human beings with renal insufficiency resulting from hemoglobinuria. From pathologic studies it appears that the cause of the renal insufficiency resulting from hemoglobinuria in dogs is obstruction of the tubular lumens with masses of pigment derived from the injected hemoglobin. These studies substantially confirm the experiments performed on rabbits by Baker and Dodds. From the Authors' Summary.

Histologic Studies in Experimental Poliomyelitis. J. A. Luhan, Arch. Neurol. & Psychiat. **37**:479, 1937.

Luhan studied the topography and evolution of poliomyelitic changes which he produced in young monkeys. The virus, which he obtained from the spinal cords of poliomyelitic monkeys, was injected into the parieto-occipital cortex of the brain. Some animals were killed before paralysis set in. The preparalytic stage, which lasted on the average one and one-seventh days, was succeeded by rapidly developing severe paralysis of the extremities. The first paralytic signs appeared in from three to fourteen days. In monkeys killed after paralysis set in, the changes in the central nervous system were vascular, meningeal and parenchymatous. The perivascular changes were infiltrations mainly with lymphocytes, mixed within the first twenty-four hours with polymorphonuclear cells and a few days later with plasma cells. In the brain, however, the latter elements could be observed even after paralysis had existed for six days. The perivascular infiltrations were mild in the meninges and in some animals were absent in the spinal cord. In contrast, they were invariably marked in the cortex, diencephalon, mesencephalon (including the substantia nigra), medulla and often also in the cerebellum. In the paralytic stage, there were also parenchymatous changes but of lesser severity and frequency. The latter changes were degeneration and liquefaction of the gray matter of the anterior horns, especially of the cervical and lumbar enlargements. No changes were observed in the olfactory bulb. In monkeys killed before the appearance of paralysis, no changes were found in the cord within two or three days after inoculation, but the thalamus, subthalamus and medulla on the side of inoculation exhibited perivascular infiltrations. In addition to the central nerve changes, animals also exhibited hyperplasia of the intestinal lymphoid structure and of the splenic follicles.

From his studies Luhan concluded that the virus of poliomyelitis is propagated by continuity within and through the nerve substance, and not through the cerebrospinal fluid or meninges. G. B. Hassin.

PRECOCIOUS PUBERTY FOLLOWING ENCEPHALITIS. F. R. FORD and H. GUILD, Bull. Johns Hopkins Hosp. **60**:192, 1937.

Two young girls following measles encephalitis showed precocious sexual development, and a boy following epidemic encephalitis showed premature development of the sex organs as well as sexual misbehavior. A survey of the literature seems to indicate that destructive lesions in the region of the pineal body and in the walls of the third ventricle may result directly or indirectly in the syndrome of macrogenitosomia praecox. Cases are cited in which this syndrome was associated not only with a tumor arising in the region of the pineal body but with a tumor of the hypothalamus. The same syndrome is known to follow inflammatory processes, such as meningitis, meningo-encephalitis and epidemic encephalitis. The information available indicates that the pineal body plays no significant role in the production of macrogenitosomia praecox, for this gland is quite normal in many cases in which this syndrome is fully developed, and destruction of the pineal body may be followed by no apparent change in the genital organs. The authors have failed to find a single instance in which definite macrogenitosomia praecox has been associated with a verified pinealoma.

FROM THE AUTHORS' SUMMARY.

THE CULTURE OF WHOLE ORGANS (THYROID GLAND). A. CARREL, J. Exper. Med. **65**:515, 1937.

A technic has been developed for the transplantation of the whole thyroid into the Lindbergh pump and for its perfusion without the occurrence of infection, embolism or other complications. The gland remains alive during the period of perfusion, which in the author's experiments lasted from three to twenty-one days. The technic is simple enough to be used as a routine procedure in physiologic or pathologic studies of the thyroid.

FROM THE AUTHOR'S CONCLUSIONS.

STUDIES ON THE SUPRARENAL CORTEX. G. A. HARROP and G. W. THORN, J. Exper. Med. **65**:757, 1937.

The effect of the administration of adrenal cortical hormone on the excretion of sodium and potassium has been studied in normal dogs. It is shown to be qualitatively similar to the effect of this hormone on adrenalectomized animals and on patients with Addison's disease. The phenomenon is proposed as the basis for a suitable method of comparative assay of extracts of adrenal cortex. The method is simple and time saving, and it uses a minimum of extract.

FROM THE AUTHORS' SUMMARY.

TOXEMIA OF PREGNANCY IN THE RABBIT. H. S. N. GREENE, J. Exper. Med. **65**:809, 1937.

The clinical manifestations and pathologic changes of a disorder associated with pregnancy in the rabbit have been described. The disorder bears a close analogy to toxemia of pregnancy in man and offers an experimental approach to the problems associated with that condition. The evidence at hand indicates that the disorder is of endogenous origin and arises from a disturbance of functions concerned in reproductive processes.

FROM THE AUTHOR'S SUMMARY.

LYMPHATICS IN OMENTAL ADHESIONS. P. H. SIMER and R. L. WEBB, Surg., Gynec. & Obst. **64**:872, 1937.

The behavior of the lymph vessels in omental adhesions has not received sufficient study. Even the presence of omental lymphatics has been questioned. In dogs, a segment of jejunal mesentery was sutured to the omental sac and the surface was irritated mechanically. Periodic examination of different dogs, followed by injections of india ink and serial sectioning, showed that lymphatic anastomoses are established between the jejunum and omentum after induction of adhesions; these channels may be demonstrable as early as eight days after operation.

WARREN C. HUNTER.

TOXICITY OF CERTAIN CHLORINE DERIVATIVES OF BENZENE, WITH SPECIAL REFERENCE TO O-DICHLOROBENZENE. G. R. CAMERON and J. C. THOMAS, J. Path. & Bact. **44**:281, 1937.

The relative toxicity of benzene and its chlorine derivatives, judged from subcutaneous administration, appears to be benzene < monochlorobenzene < p-di chlorobenzene < o-dichlorobenzene > trichlorobenzene > tetrachlorobenzene > hexachlorobenzene. Substitution of chlorine in the benzene ring therefore increases the toxicity with each successive chlorine atom up to two. Further chlorination reduces the toxicity. There is some evidence, also, that the structural position of the chlorine atoms influences the toxicity of the molecule, for o-chlorobenzene is more toxic than p-dichlorobenzene. Since exposure of small laboratory animals to o-dichlorobenzene in the concentrations reached during and after fumigation of a room produces slight renal damage, often severe necrosis of the liver and occasionally death, it seems advisable that caution be exercised in the use of the lower chlorine substitution products of benzene. The authors consider it inadvisable that human beings should be exposed to the influence of these products, even in low concentrations, for any length of time.

FROM THE AUTHORS' SUMMARY.

ACTION OF HYPOPHYSIAL EXTRACTS AND OF THYROXIN ON HEREDITARY HYPOPHYSIAL INFANTILISM IN MICE. T. KEMP and L. MARX, Acta path. et microbiol. Scandinav. **14**:197, 1937.

This is a continuation of a previous report in which the development of the dwarfed mouse was described. The anterior lobe of the hypophysis of the dwarfed mouse was hypoplastic, and the eosinophilic cells were few or absent. The thymus was smaller than in the normal animal. It became hyperplastic after brief administration of the growth hormone, the lactogenic hormone and the thyrotropic hormone of the hypophysis and of thyroxin, but hyperplasia ceased if treatment continued. The thyroid was equally hypoplastic. The growth hormone caused increase of growth; the action of thyrotropic hormone was not constant. The growth hormone stimulated the growth of the parathyroids. The medulla of the adrenal was normal, but the cortex was not fully developed in the untreated animal. The growth hormone caused hypertrophy of the medulla and still further thinning out of the cortex. Combining the growth hormone with thyroxin stimulated normal growth of the cortex. The pancreas showed progressive rapid atrophy, that was prevented by administration of the growth hormone and thyroxin, the former stimulating the development of the islands and the thyroxin causing their decrease. The growth of the whole animal was greatly influenced by the growth hormone, and normal size was reached after a treatment of from two to three months. The sexual functions became normal in the male animals but were less influenced in the females. The action of the other hormones was evident but not so striking when given alone, but when they were combined with the growth hormone the gain of weight was greatest.

I. DAVIDSOHN.

Pathologic Anatomy

STRUCTURE OF THE SMALL CEREBRAL ARTERIES AND THEIR CHANGES WITH AGE. A. B. BAKER, Am. J. Path. **13**:453, 1937.

The average small cerebral artery differs in structure from similar-sized vessels elsewhere in the body in that it contains within its media a relative paucity of both elastic and muscle tissue and a predominance of collagenous fibers. The very small cerebral arteries are composed almost strictly of collagenous tissue and may appear as cerebral fibrosis. With advance of age the elastica interna of the cerebral arteries becomes reduplicated and frequently loses its normal tinctorial properties. The media undergoes rapid fibrosis. It frequently shows hyalinization and more rarely calcification of all of its elements.

FROM THE AUTHOR'S CONCLUSIONS.

HISTOPATHOLOGY OF IDIOPATHIC THROMBOCYTOPENIC PURPURA HAEMORRHAGICA. D. A. NICKERSON and D. A. SUNDERLAND, Am. J. Path. **13**:463, 1937.

Differential cell counts have shown no fundamental differences in the bone marrow in idiopathic thrombocytopenic purpura haemorrhagica, various other forms of purpura haemorrhagica and normal conditions. Megakaryocyte counts in all forms of purpura show no constant variation except predominance of the functionally active forms. Under proper technical conditions active platelet formation was demonstrated in the bone marrow in one case of idiopathic and in one case of secondary thrombocytopenic purpura haemorrhagica. A fairly uniform microscopic picture in the spleen and the presence of megakaryocytes in the various organs in idiopathic thrombocytopenic purpura haemorrhagica are reported. The theories of pathogenesis are presented, and evidence is introduced that thrombocytolysis is the cause of thrombocytopenia. It is suggested that this in turn causes compensatory hyperplasia of the megakaryocytes in the bone marrow. The literature on the histology of idiopathic thrombocytopenic purpura haemorrhagica is briefly reviewed.

FROM THE AUTHORS' SUMMARY.

PERIVENOUS CHANGES IN ACUTE ENCEPHALITIS ASSOCIATED WITH VACCINATION, VARIOLA AND MEASLES. K. H. FINLEY, Arch. Neurol. & Psychiat. **37**:505, 1937.

From a study of fourteen cases of postvaccinal encephalitis, three of postmeasles encephalitis and one of encephalitis associated with variola, Finley comes to the conclusion that the extra-adventitial infiltrations are glial (Microglia is evidently classified by Finley as glia. G. B. H.) and perivenous, not periarterial. He states that "small and large veins were involved but the medium and large vessels presented a marked glial reaction." These were frequently surrounded by a glial collar, which was not the case with the small branches of veins, and for this reason it is stated that the latter had "no or a minimal glial reaction." The local tissue reaction showed exclusively around the veins and was not limited to the white substance of the central nervous system. Lesions were observed in the corpus striatum, for instance, in 90 per cent of cases, though this structure is "almost free from myelin."

G. B. HASSIN.

SYPHILITIC ARACHNOIDITIS OF THE OPTIC CHIASM. L. HAUSMAN, Arch. Neurol. & Psychiat. **37**:929, 1937.

On the basis of a study of five cases of the so-called optic chiasm syndrome (bilateral optic atrophy combined with heteronymous hemianopia) Hausman emphasizes the importance of syphilitic arachnoiditis as one of its causes. As syphilitic arachnoiditis is much rarer than the nonsyphilitic chronic cisternal arachnoiditis, it is usually not recognized, and some other parasellar lesion or tabes dorsalis is diagnosed instead. In one of the five cases extensive adhesions around the chiasm were removed, and the patient's vision improved considerably. In another case, instead of optic atrophy, there was marked papilledema without signs of increased intracranial pressure or evidences of concomitant hydrocephalus, but the visual fields were concentrically contracted. Necropsy revealed intensive arachnoiditis of the interpeduncular space, which was filled with mucopurulent exudate and exhibited perichiasmal gummatous syphilitic meningitis with typical syphilitic vascular changes and infiltrations with lymphocytes, plasma cells and giant cells. In addition, a gumma in the right parietal lobe was found. As the Wassermann and Kahn tests are practically always positive in such cases, Hausman suggests that antisyphilitic treatment should be instituted before an operation for relief from the failing vision is recommended.

G. B. HASSIN.

THE PATHOLOGIC CHANGES IN THE LYMPH NODES IN INFECTIOUS MONONUCLEOSIS. H. DOWNEY and J. STASNEY, Folia haemat. **54**:417, 1936.

A study of histologic sections and of imprints of biopsy specimens of lymph nodes in eight cases of infectious mononucleosis at different stages of the disease

showed great variation depending not only on the stage of the illness but even on the location in the node. The hyperplasia involved the lymphocytes to such a degree that occasionally the condition resembled lymphatic leukemia; it also involved the reticulum cells. Among the lymphocytes, cells with basophilic cytoplasm similar to those that are found in the circulation were seen, and frequently a quantitative correlation was apparent between their number in the nodes and their number in the blood. Nodules of swollen, rounded reticulum cells were frequent. Transitions from the reticulum cells to lymphocytes and from the latter to plasma cells could be followed. According to Downey and Stasney, the hematologic, histologic and cytologic observations in infectious mononucleosis point to a leukemoid reaction to an infective toxic-producing lymphotropic agent.

I. DAVIDSOHN.

ESTIMATION OF TOTAL QUANTITY OF BLOOD AT NECROPSY. C. BÖSS, Virchows Arch. f. path. Anat. **297**:305, 1936.

The total quantity of blood obtainable at necropsy was determined by the method of Rössle in over 1,500 necropsies. By the method named the blood from the heart and that from the lungs are measured separately. The rest of the blood is allowed to flow into the empty thoracic cavity and is measured together with all clots. This is a routine procedure in Rössle's institute, from which this report comes. It is admittedly crude and does not pretend to give the measure of all the blood in the body. But Böss thinks that by correlating the quantity of blood at necropsy with the age, sex, height and body weight and with the disease that was the principal cause of death, useful information was obtained. The average quantity was 5.8 cc. per kilogram of body weight. In deaths associated with cardiac decompensation the quantity per kilogram was increased, whereas in peripheral circulatory insufficiency (shock and collapse) the quantity was decreased. In various acute infectious diseases the quantity per kilogram was decreased, which is indicative of peripheral vascular insufficiency. In death of central (cerebral) origin the quantity was about normal, whereas in asphyxial death the quantity was high, a finding that he believes may be of forensic value. In the arterial hypertension of secondary contracted kidney and malignant nephrosclerosis with severe renal insufficiency (pale hypertension) low values were obtained as compared with those in hypertension without renal insufficiency (red hypertension of Vollhard). In acute hemorrhagic nephritis the values were high and in pyelonephritis low. Determination of the quantity of the blood should be a regular necropsy procedure, like the weighing and measuring of organs.

O. T. SCHULTZ.

TRANSPOSITION OF THE PULMONARY VEINS INTO THE RIGHT AURICLE. A. NAGEL, Virchows Arch. f. path. Anat. **297**:343, 1936.

In a child dead at the age of 48 days the pulmonary veins united into a single pair of vessels which opened into the coronary sinus and therefore emptied their blood into the right auricle. The right side of the heart was greatly hypertrophied, the left side appearing as an appendage to the right. There were no other anomalies. The foramen ovale was slitlike, and the ductus arteriosus was closed. Closure of the ductus, in spite of the fact that most of the blood of the body was within the lesser circulation, is interpreted as evidence that changes in the relative pressures within the aorta and pulmonary artery were not a factor in the closure.

O. T. SCHULTZ.

ARCHITECTURE OF THE LYMPHATICS OF THE CAPSULE OF THE LIVER. H. HASS, Virchows Arch. f. path. Anat. **297**:384, 1936.

In this study, based on human necropsy material, an injection of opaque material was made into the capsular lymphatics of the liver. The physical properties of

the material used were such that the finest lymphatics were filled but the material did not pass into the tissue spaces of the normal liver. The success of the method is attested by the excellent photographic illustrations that accompany the text. In the normal liver the flow of the lymph stream is toward the diaphragmatic surface. The larger lymphatic trunks break up within the capsule into a network of fine capillaries. In acute generalized infections the fluid content of the capsule is increased and the injected material passes into the tissue spaces. The direction of lymph flow is dependent on the density of the connective tissue of the liver and on the size and number of the capsular lymphatics. In cirrhosis of the liver many of the finer lymphatics are obliterated, and the connective tissue of the liver is denser than normal. Hence the normal direction of lymph flow is altered and a larger proportion of the fluid passes through the capsule into the peritoneal cavity than is the case in the normal liver.

O. T. SCHULTZ.

WIDESPREAD FAT NECROSIS IN CARCINOMA OF THE PANCREAS. M. TITONE, Virchows Arch. f. path. Anat. **297**:416, 1936.

Necropsy of a man who died at the age of 56 years of carcinoma of the pancreas revealed unusually widespread fat necrosis. Necrosis was present not only in the fat about the pancreas and of the mesentery but also in the retroperitoneal fat and in the subcutaneous fat, especially of the arms and thighs. In the latter situations the necroses were extensive and undermined the skin. The necroses were of various ages; those of the subcutaneous tissue were most recent. Necrosis was evident also in tissues other than adipose tissue, namely, the lungs, muscles, bone and cartilage. Metastases were present in the liver, but no tumor cells could be found in or near the areas of necrosis.

O T. SCHULTZ.

GENERALIZED RHEUMATIC CHONDROMALACIA. F. ALTHERR, Virchows Arch. f. path. Anat. **297**:445, 1936.

This is a detailed study and discussion of a unique case. A boy of 14 years became ill nine months before his death with acute tonsillitis. This was followed by nonpurulent febrile polyarthritis. Softening of the laryngeal cartilages and collapse of the larynx and trachea necessitated tracheotomy. The cartilage of the nose and ears also became softened. Death was due to generalized miliary tuberculosis, which had its origin in a fresh caseous lesion of the upper lobe of a lung. Necropsy revealed marked softening of all the cartilaginous tissues of the body. In the periarticular tissues there was observed the fibrinoid degeneration of early active rheumatic disease, and in the myocardium, Aschoff bodies (rheumatic granulomas). The cartilaginous tissues revealed a combination of degenerative, resorptive and inflammatory changes. The degenerative changes consisted in fibrinoid swelling, hyalinization, asbestos-like degeneration and splitting of the ground substance and disappearance of cartilage cells. Resorption of degeneration products occurred by means of an inflammatory granulation tissue which invaded the cartilage from the surrounding perichondrium or joint capsule. Localized small areas of new formation of cartilage and bone were seen. The author concludes that the generalized disease of the cartilaginous system was part of the rheumatic process, the widespread involvement of the cartilage having perhaps a constitutional basis.

O. T. SCHULTZ.

HEMORRHAGE INTO THE POSTERIOR PART OF THE BRAIN STEM AS A CAUSE OF SUDDEN DEATH. O. BERNER, Virchows Arch. f. path. Anat. **297**:495, 1936.

Berner briefly discusses a sudden death due to multiple small hemorrhages in the floor of the fourth ventricle. He can offer no explanation of the hemorrhages.

O. T. SCHULTZ.

ACTIVATION OF THE MESENCHYME IN INFLAMMATION. C. CORONINI, Virchows Arch. f. path. Anat. **297**:523, 1936.

Coronini considers the reaction of the mesenchyme, and especially of its capillary endothelium and connective tissue, to be the earliest and most characteristic feature of the inflammatory process. This reaction he terms embryonal activation, a process which leads to the formation of young cells with the multiple potencies of embryonic cells. The usual and what may be termed the normal mesenchymal reaction to an inflammatory stimulus may be modified and intensified by a hyperergic state of the tissues. The author postulates that a still further modification of the inflammatory process, through causes as yet unknown, might lead to continued proliferation with loss of the potentiality for differentiation and to leukemia or sarcoma on the basis of what was originally an inflammatory process.

O. T. SCHULTZ.

THE PATHOLOGIC ANATOMY OF HUMAN KALA-AZAR. C. H. HU, Chinese M. J., supp., 1936, p. 1.

A study of thirty-one cases of kala-azar coming to autopsy revealed the fact that although the parasitization and great increase in number of the cells of the reticulo-endothelial system are the most important characteristic feature of the pathologic anatomy of this disease, there are a few changes which have not been sufficiently recognized. First, there is the frequent extramedullary formation of blood on the dural surface of the calvarium with secondary growth of new bone on the inner surface, in the corresponding areas. Second, there is myelocytic hyperplasia, which is especially striking after the parasites and their containing cells have disappeared. The young myeloid cells, especially the myelocytes, are greatly increased in number whereas the metamyelocytes and leukocytes are decreased; the normoblasts are in most cases either not greatly increased or even probably decreased in number. Third, there is an increase in the number of plasma cells in the spleen and bone marrow, which may remain for a considerable time after the disappearance of the infested cells. A positive diagnosis of kala-azar cannot be made without the finding of parasites. As a result of this study certain anatomic criteria are found, which, when considered together, will make the diagnosis of kala-azar reasonably, though not absolutely, certain in the absence of parasites.

FROM THE AUTHOR'S SUMMARY.

Pathologic Chemistry and Physics

THE MINERAL CONTENT OF VARIOUS CEREBRAL LESIONS AS DEMONSTRATED BY THE MICRO-INCINERATION METHOD. L. ALEXANDER and A. MYERSON, Am. J. Path. **13**: 405, 1937.

This article contains a summary of the results of an extensive study of cerebral structures under various conditions by means of micro-incineration, the many details of which should be sought in the original.

BLOOD NITRITE. E. J. STIEGLITZ and A. E. PALMER, Arch. Int. Med. **59**: 620, 1937.

By the use of alphanaphthylamine and disodium betanaphthylamine-6,8-disulfonic acid the nitrite ion was found in small amounts in the blood, saliva and sweat but not in the spinal fluid or normal urine of man. The nitrite of the blood was found to originate from bacterial reduction of food or drug nitrates in the lower bowel or from reduction of nitrates in the tissues. Nitrite relaxes smooth muscle, especially arteriolar muscle. A correlation may exist between disturbed nitrite metabolism and abnormalities of arterial tension.

FREDERICK STENN.

AMINO-ETHYL PHOSPHORIC ESTER FROM TUMOURS. E. L. OUTHOUSE, Biochem. J. **30**: 197, 1936.

The author describes the isolation of a phosphoric ester from bovine malignant tumors. The purified ester was found to be identical with synthetic amino-ethyl phosphate.

R. J. LEBOWICH.

URINARY PORPHYRINS IN DISEASE. K. DOBRINER, J. Biol. Chem. **113**:1, 1936.

Porphyrins were isolated from the urine and purified, and each was identified by the acid number, absorption spectrum, crystal form and melting point of the methyl ester. The porphyrin excreted in urine in Hodgkin's disease, in atrophic cirrhosis of the liver and in catarrhal, obstructive and hemolytic jaundice was coproporphyrin I; it was also present in a small amount in normal urine. Coproporphyrin III was excreted in the urine in pigmentary cirrhosis of the liver and in tumor of the liver.

R. J. LEBOWICH.

RELATIONS BETWEEN POISONING BY SELENIUM AND PELLAGRA. R. DE R. BARONDES, Presse méd. **45**: 188, 1937.

Because of the close identity in pathologic and symptomatic features between black tongue in dogs and pellagra in man on one hand, and selenium poisoning, on the other, it is suggested that selenium is a cause of these diseases. An analysis of food and soil for this element in pellagrous areas is advised.

FREDERICK STENN.

TOPOGRAPHIC DISTRIBUTION OF POTASSIUM AND CALCIUM IN NORMAL AND PATHOLOGIC TISSUES. W. SCHULZE and H. ZSCHAU, Frankfurt. Ztschr. f. Path. **48**: 51, 1935.

The method of Macallum was used exclusively for this investigation. Blocks of various tissues were fixed in a neutral 10 per cent dilution of solution of formaldehyde and embedded in gelatin. The distribution of calcium and potassium was found to adhere to certain rules. Potassium and calcium were present in all tissues except nonpathologic elastic fibers. The nuclei of cells generally contained more potassium than the cytoplasm, and a relative increase of potassium was noted where the tissue reaction was either relatively or absolutely acid, as in the cytoplasm of epithelial cells, axis-cylinders of peripheral nerves, muscle fibers, bone and cartilage. Calcium was relatively increased in the cytoplasm of connective tissue cells, in the perinuclear areas of other cells and between cells in general. Mitotic figures showed potassium and calcium in the chromatin loops. A marked increase in calcium was also seen in the cytoplasm of lipoid cells and goblet cells. Generally speaking, calcium seems to function as a material for cell borders and tissue framework.

In wounds, during the first phase of healing, a phase in which the reaction of the tissue is acid, calcium and potassium are increased. Later potassium predominates. In repair, or the reconstructing phase, calcium predominates. Inflammatory processes follow similar rules. In necrotic tissue potassium is decreased and calcium is increased. Normal endothelial cells contain a relatively large amount of calcium. Endothelial cells found in prethrombotic and thrombotic vessels reveal a decreased amount or absence of calcium. Benign tumors reveal a distribution of calcium and potassium similar to that in normal tissue. In malignant tumors the calcium-potassium equilibrium is destroyed.

OTTO SAPHIR.

Microbiology and Parasitology

SOME CHEMICAL PROPERTIES OF AN ESSENTIAL GROWTH FACTOR FOR PATHOGENIC BACTERIA. F. SAUNDERS, I. I. FINKLE, L. STERNFELD and S. A. KOSER, J. Am. Chem. Soc. **59**:170, 1937.

Many plant and animal tissues contain a substance which is essential for the growth of certain pathogenic bacteria. This substance has been purified partially, and the chemical properties of the impure preparations have been studied. The growth factor is soluble in water, methanol, ethanol and phenol, but it is insoluble in the higher alcohols, ether, benzene and chloroform. The activity is not appreciably affected by autoclaving, aeration or boiling or by oxidizing agents, such as 3 per cent hydrogen peroxide, or by ammoniacal silver, and is only slightly affected by bromine in the cold. Treatment with acetic anhydride caused only slight loss of activity. The growth factor probably does not contain sulfur. It is not inorganic since it is destroyed by both wet and dry ashing.

FROM THE AUTHORS' SUMMARY.

PRODUCTION OF HEMORRHAGIC-NECROTIC SKIN LESIONS IN THE RABBIT BY HAEMOPHILUS INFLUENZAE AND HAEMOPHILUS PERTUSSIS. E. WITEBSKY and H. SALM, J. Exper. Med. **65**:43, 1937.

Haemophilus influenzae injected intradermally into the abdominal wall of the rabbit induces inflammation, frequently combined with development of a central pustule. Haemophilus pertussis injected similarly causes a bluish violet discoloration of the area of skin involved, which undergoes slight hemorrhagic-necrotic changes within a few days. Living H. influenzae injected intravenously twenty-four hours after intradermal inoculation of living H. influenzae is able to transform the respective areas of skin into severe hemorrhagic necrotic lesions within from three to five hours. Heat-killed H. influenzae, if injected intravenously, may produce hemorrhagic-necrotic lesions in areas previously prepared with living or heat-killed H. influenzae. H. pertussis, if injected intravenously, may cause, though perhaps to a lesser extent, hemorrhagic-necrotic lesions in areas of skin that twenty-four hours previously had received injections of H. influenzae. The normal course of an infection of rabbit skin with H. pertussis is not, or not essentially, influenced by intravenous reinjection of living or killed H. influenzae or H. pertussis. The agar washing filtrate of B. typhosus, if injected intravenously, can produce hemorrhagic necrotic lesions in rabbit skin prepared intracutaneously with living as well as with heat-killed H. influenzae. The intravenous injection of B. typhosus agar washing filtrate has no influence on areas prepared with H. pertussis. Conversely, H. influenzae as well as H. pertussis, if injected intravenously, are able to produce hemorrhagic necrotic lesions in rabbit skin prepared twenty-four hours previously with B. typhosus agar washing filtrate. The effectiveness of suspensions of H. influenzae apparently is confined to the bacteria themselves rather than to the supernatant fluids. This does not exclude the possibility of producing effective exotoxins under special experimental conditions.

FROM THE AUTHORS' SUMMARY.

INHERITANCE OF RESISTANCE OF MICE TO ENTERIC BACTERIAL AND NEUROTROPIC VIRUS INFECTIONS. L. T. WEBSTER, J. Exper. Med. **65**:261, 1937.

Under the conditions specified, there may be selected promptly from a hybrid stock of mice, of which from 40 to 50 per cent die on being given a standard dose of Bacterium enteritidis or of the virus of encephalitis B (St. Louis), lines in which as high as 95 per cent and as low as 15 per cent succumb. Three lines—one susceptible to invasion by both bacteria and virus, one susceptible to bacterial infection but resistant to virus invasion, and one resistant to infection by bacteria but

susceptible to invasion by virus—are regarded as remaining relatively stable after approximately twelve generations of selection and brother to sister or line inbreeding. Crossing susceptible with resistant lines and testing F_1, F_2, F_3 and backcross progeny resulted in mortality percentages in the neighborhood of those expected on the basis that resistance to Bact. enteritidis and to the virus of encephalitis B is inherited independently on the basis of a single factor, with resistance dominant over susceptibility. A bacteria-resistant, virus-resistant line is being developed from a cross between bacteria-susceptible, virus-resistant and bacteria-resistant, virus-susceptible lines. All selected lines proved uniformly susceptible to a strain of mouse passage rabies virus.

FROM THE AUTHOR'S SUMMARY.

CATAPHORETIC VELOCITY OF THE TYPHOID BACILLUS. R. M. WATROUS, J. Infect. Dis. **60**:47, 1937.

The cataphoretic velocity of one strain of the typhoid bacillus in a particular menstruum of constant ionic concentration has been shown to vary with the age of the culture in three mediums, decreasing in agar and broth and rising in K medium. The cataphoretic velocity of the same organism under these conditions is shown to be different for cultures of the same age grown in different mediums, being greater in broth than in agar.

FROM THE AUTHOR'S SUMMARY.

CALIFORNIA RELAPSING FEVER. M. D. BECK, J. Infect. Dis. **60**:64, 1937.

In California, where relapsing fever is endemic, 106 cases of the disease were reported from 1921 through 1935. There are nine foci of infection in the mountainous districts over 5,000 feet in elevation, the important ones being Big Bear Lake, Lake Tahoe and Packer Lake.

Thirteen strains of spirochetes resembling Borrelia recurrentis were isolated from rodents in the field. In the districts surveyed only chipmunks and Tamarack squirrels (Sierra chickaree) were found to harbor the spirochetes.

In mice periods of latency as long as four months are demonstrable.

The spirochetes, both human and animal strains, show remarkable resistance to freezing, to being shipped uniced in the clot and in the tissues and to being kept viable in defibrinated sheep blood for at least six months.

Hyperimmune guinea-pig serums have been produced with strains of spirochetes from chipmunks and Tamarack squirrels (Sierra chickaree). Protection was obtained with homologous and heterologous rodent strains, and in one instance protection was demonstrated with antiserum produced with a rodent strain against inoculation with a human strain of spirochetes.

The rodent and human strains were found to be morphologically identical and similar in pathogenicity for laboratory animals. These strains are undoubtedly the same, since the rodent strains are directly transmissible to man.

FROM THE AUTHOR'S SUMMARY.

THE DISTRIBUTION OF BRUCELLA MELITENSIS VARIETY MELITENSIS IN THE UNITED STATES. A. C. EVANS, Pub. Health Rep. **52**:295, 1937.

Human infection with Brucella melitensis variety melitensis has long been known in southwestern United States. In the literature are records of occasional human and bovine infection with the melitensis variety in various other sections of the United States.

A review is given of the reports in which the grouping of Brucella according to serologic reactions is correlated with the grouping according to bacteriostatic reactions. Except in certain restricted localities, there is a low percentage of atypical strains with regard to which the groupings according to the two systems do not agree. Of 259 American strains which have been studied by various

investigators, only 19 (7.3 per cent) were atypical. Hence, although agglutinin absorption tests will not classify an individual strain of Brucella in the abortus-suis or melitensis group with absolute certainty, collected data will give information as to the types of infection in a given locality.

The results of this study indicate that infection of human beings with the melitensis variety in the three survey areas occurred as follows: in Charlotte, N. C., in 5 of 7 cases of brucellosis, or 71.4 per cent; in San Antonio, Texas, in 6 of 10 cases, or 60 per cent; in Kansas City, Kan., in 2 of 10 cases, or 20 per cent.

FROM THE AUTHOR'S SUMMARY.

INTRA-ENDOTHELIAL BODIES IN RABIES. A. C. COLES, J. Path. & Bact. **44**:315, 1937.

In rabies the endothelial cells of small blood vessels in the brain contain small stainable particles which have the appearance of being parasites.

FROM THE AUTHOR'S SUMMARY.

BACTERIOPHAGE OF LACTIC STREPTOCOCCI. H. R. WHITEHEAD and G. J. E. HUNTER, J. Path. & Bact. **44**:337, 1937.

Phages appear to arise spontaneously in cultures of lactic strains of Streptococcus under certain conditions of growth in milk. A resistant culture prepared by the action of the primary phage in a sensitive strain alternately suffers attack by a further phage, quite distinct from the original one. A series of resistant cultures and secondary phages can thus be obtained for each type of Streptococcus. That the phages gain access to cultures by contamination from outside sources is unlikely in view of the specific relationships which exist between phages and organisms and the consequent multiplicity of phages which it would be necessary to postulate as existing constantly in the surroundings of the cultures. It is therefore suggested that the results support the theory that phage is a product of the organism.

FROM THE AUTHORS' SUMMARY.

A NEW RICKETTSIA INFECTION; RICKETTSIA WEIGLI, NOV. SP. H. MOSING, Arch. Inst. Pasteur de Tunis **25**:373, 1936.

There occurred during 1934 an epidemic among eighteen employees at the Institute of Biology, Levow. This group included nearly all of approximately forty members of the institute, engaged in the manufacture of Weigl vaccine, who were engaged in daily feeding of numerous lice. The period of incubation appeared to be from ten days to between three and six weeks; it was even two months in experimental infection on the author. A sharp rise in temperature (39 to 41 C. [102.2 to 105.8 F.]) was followed by a sudden drop, but as many as seven recurrent rises occurred, most often three. Lymphocytes dropped to 10 per cent during febrile attacks, but lymphocytosis was noted between attacks. Occipital headaches occurred regularly. No exanthem was noted. The difference between this disease and typhus was marked, although several persons had had typhus and all had been vaccinated. Dr. Weigl, director of the institute, had typhus several times during a period of some years. Vaccination is known not to give absolute immunity. Every check, however, seemed to rule out typhus. Rickettsia Weigli was noteworthy for the larger size of its bodies and because these were extracellular. The Weil-Felix reaction was negative, but agglutination to the titer 1:100 was noted with suspensions of the rickettsia. The patients' blood as tested by louse infection was infectious, particularly after the first febrile period, and remained so long after clinical recovery. The urine was also infectious.

M. S. MARSHALL.

Immunology

PURIFIED PROTEIN DERIVATIVE OF TUBERCULIN (P P D). E. R. LONG and F. B. SEIBERT, Am. Rev. Tuberc. **35**:281, 1937.

With a small number of exceptions, patients with clinical tuberculosis react to the purified protein derivative of tuberculin and the great majority react to the first, or smaller, of the standard doses (viz., 0.00002 mg.). The exceptions are for the most part patients with acute tuberculosis or seriously ill patients with chronic tuberculosis of long standing. A small number of subjects with calcified tuberculous lesions from a first infection do not react to the purified protein derivative or other forms of tuberculin. There is evidence that some of the reactions to the second dose of purified protein derivative in cases in which the reaction to the first dose is negative are nonspecific. This applies equally to other forms of tuberculin. Reactions to the second dose are elicited equally well by large doses of analogous protein derivatives from other acid-fact bacteria. This appears to be a nonspecific effect, because small doses of the latter protein derivatives do not cause reactions in subjects highly sensitive to purified protein derivative of the tubercle bacillus. Further evidence of nonspecificity, not yet entirely clear, appears in the fact that the increment of reactions gained by the second dose is approximately constant in terms of the total number of subjects tested, regardless of the number reacting to the first dose. Some advance has been made in selecting an intermediate dose; this varies for different parts of the country and for different social groups. Figures obtained from intermediate doses will not be suitable for statistical comparison in different parts of the country. Dilutions of purified protein derivative, like dilutions of other forms of tuberculin, lose strength on standing. The loss is probably partly due to mild bacterial contamination occurring in spite of antiseptic preservation. However, for practical purposes it seems that the standard dilutions can be safely kept in the icebox for three days.

H. J. CORPER.

COMPLEMENT FIXATION FOLLOWING B C G VACCINATION. G. B. REED and B. G. GARDINER, J. Immunol. **31**:471, 1936.

Serum of rabbits inoculated repeatedly with S tubercle bacilli reacted with the homologous antigen in higher dilutions than with R bacilli. Serum from patients with active tuberculosis reacted similarly, while serum from moribund patients and patients whose condition was chronic reacted with both antigens to the same extent. The serum of eleven children inoculated with BCG intracutaneously fixed almost identical amounts of complement with S and R antigens This suggests absence of the specific S antigenic substance from BCG vaccine.

I. DAVIDSOHN.

IMMUNOLOGIC STUDIES WITH PURIFIED SERUM PROTEINS BEARING ON THE UNITARIAN THEORY OF ANTIBODIES. E. DELVES, J. Infect. Dis. **60**:55, 1937.

Agglutination and phagocytosis of collodion particles coated with purified human albumin and human pseudoglobulin and complement fixation with these antigens in solution can be obtained with their homologous precipitin antiserums. When the precipitin was entirely removed by absorption with homologous antigen, the agglutinins and opsonins were completely removed. The complement-fixing power was completely removed from the two albumin antiserums and was greatly reduced in the two pseudoglobulin antiserums.

These results give indirect evidence that the antigen-antibody reactions of agglutination, precipitation, complement fixation and opsonification may be merely different manifestations of a single antibody when that antibody is produced by a purified antigen. Conflicting theories as to the multiplicity or singleness of antibodies have arisen in part because complex antigens have been used in much of the work.

FROM THE AUTHOR'S SUMMARY.

THE FREI TEST. W. H. CONNOR, E. A. LEVIN and E. E. ECKER, J. Infect. Dis. **60**:62, 1937.

The Frei test appears to be specific for lymphogranuloma venereum. Other venereal, infectious and miscellaneous diseases give negative reactions. A positive reaction has been obtained as long as thirty-nine years after infection. Repeated testing in nonreactive patients does not appear to raise cutaneous allergy to the Frei antigen. In this series the Frei test was uniformly positive if the bubo had reached a duration of forty days. The latent period preceding the Frei reaction varies from seven to thirty-nine days after the onset of the bubo. The pus may contain antigenic substances prior to the development of a cutaneous reaction.

FROM THE AUTHORS' SUMMARY.

INHERITANCE OF ALLERGIC DISEASE. A. S. WIENER, I. ZIEVE and J. H. FRIES, Ann. Eugenics **7**:141, 1936.

As the authors of this article point out, there seems to be no unanimity of opinion as to the mode of transmission of allergic disease. According to Cooke and Vander Veer and Spain and Cooke, allergy is transmitted as a simple mendelian dominant. In direct contrast, Adkinson maintains that her findings favor a recessive mechanism. Furthermore, Richards and Balyeat suggest that the condition is inherited as a "partial dominant." An analysis of the authors' studies of 66 complete families with 250 children, as well as of data already published, failed to support either the simple dominant or the recessive theory. The theory that allergy is transmitted as a simple mendelian dominant does not explain why more than half of the pedigrees of both parents are normal. The theory that the mechanism is recessive is refuted by the existence of pedigrees in which both parents are affected yet some of the children are normal. The authors present for consideration a new theory of heredity of allergic disease. According to this theory, allergic disease is transmitted by means of a single pair of allelomorphic genes, *H* and *h*; *h* determines the allergy, and *H* is the contrasting normal gene. Three different genotypes are possible:

1. Genotype *HH*—pure normal.

2. Genotype *hh*—pure allergic. Persons of this genotype show symptoms of allergic disease before the age of 10 years.

3. Genotype *Hh*. The majority of persons of this type remain apparently normal throughout life although they transmit the abnormal gene *h*. The rest, depending on environmental conditions, will manifest allergic diseases, but the symptoms will not appear until after puberty. The existence of two sorts of allergic persons is supported by the bimodal distribution when cases are classified according to age of patient or onset. Moreover, statistical analysis of the authors' own findings in studies of families and of data previously published lends additional support to the theory. One finding that remains to be explained is the excess of males over females among those in whom allergic disease develops before puberty.

A. S. WIENER.

HYPERERGIC INFLAMMATORY REACTION OF THE GASTRO-INTESTINAL TRACT. H. KAISERLING and W. OCHSE, Virchows Arch. f. path. Anat. **298**:177, 1936.

Contrary to the accepted view that a hyperergic inflammatory reaction can be elicited in any organ of the sensitized animal by proper local injection of the specific antigen, Scholer claimed that no such reaction could be evoked in the small intestine of the sensitized rabbit. Kaiserling and Ochse sensitized rabbits by repeated subcutaneous injections of horse or swine serum. At the proper interval after the last sensitizing injection the gastro-intestinal tract was exposed and the activating dose of the corresponding antigen was injected into the wall

of the tract at various levels. The injections were submucous, subserous or interstitial. The animals were killed at intervals of from two hours to three days after the shock injection and the tissues examined grossly and microscopically. The characteristic hyperergic reaction was set up at all levels of the gastro-intestinal tract.

O. T. SCHULTZ.

IMMUNOLOGIC STUDIES ON THE PNEUMOCOCCUS. B. F. CHOW and H. WU, Chinese J. Physiol. (no. 2) **11**:139, 155, 163, 169 and 183, 1937.

To pneumococcus type I antiserum (horse or rabbit) homologous polysaccharide was added. The resulting precipitate was dissolved with seventieth-normal sodium hydroxide and reprecipitated with seventieth-normal hydrochloric acid. The recovered antibody protein had the power of causing agglutination and precipitation as well as protecting mice from lethal doses of type I pneumococci to a higher titer than the original immune serum. Pneumococcus type I antibody purified according to the ammonium sulfate method did not give as high a titer. Adequate recovery of the antibody from the immune precipitate was dependent on careful adjustment of the p_H over a period of several hours. The same method was employed for the isolation of antibody proteins from the immune agglutinate. A type I pneumococcus vaccine was added to antipneumococcus type I serum of horse or rabbit. The resulting agglutinate was washed and dissolved in seventieth-normal sodium hydroxide and reprecipitated in seventieth-normal hydrochloric acid. The recovered antibody proteins had sixteen times higher agglutinative and protective activity than those of the original serum. Immunologically, pure precipitin of rabbit origin caused agglutination, protected mice from a lethal dose of type I pneumococci, produced passive anaphylaxis and fixed complement besides precipitating its homologous polysaccharide, thus confirming the unitarian hypothesis of antibodies. The method of Chow and Wu is applicable to the isolation of antibodies from antipneumococcus type II and type III immune serums. Chow and Wu report isolation of a new fraction of the protective antibody from antipneumococcus type I immune rabbit serum. To antipneumococcus type I rabbit serum previously absorbed with the homologous polysaccharide a suspension of R organisms of Pneumococcus type I was added, and to the supernatant fluid a suspension of S organisms was added. The resulting agglutinate was washed and the antibody recovered by applying the alkali-acid treatment. This new fraction was found to be immunologically different from the antipolysaccharide precipitin. It agglutinated specifically and had a protective titer four times greater than the antipolysaccharide antibody.

FREDERICK STENN.

Tumors

ISOLATION OF PURE STRAINS OF CELLS FROM HUMAN TUMORS. H. PINKUS, Am. J. Cancer **29**:25, 1937.

The tumor which because of its clinical importance has been the most common object of study by former students, i. e., the squamous cell carcinoma, is probably the least suitable for tissue culture. Rapidly growing tumors, forming dense areas in vitro, offer the best chance of success. Spontaneous malignant growths are composed of a genetically inhomogeneous and labile cell material. Inhomogeneity and lability differentiate spontaneous tumors from transplantable malignant growths, the elements of which have been thoroughly stabilized by selection. Inhomogeneity and lability account for a great part of the difficulties encountered in the cultivation of the tumors of man. Careful selection of specimens and a technic taking into account points 3 to 5 in the original article will likely make possible permanent cultivation of pure strains of human malignant cells.

FROM THE AUTHOR'S CONCLUSIONS.

PROGNOSTIC VALUE OF THE MITOSIS COUNT IN BIOPSIES OF LYMPHOSARCOMA. A. E. CASEY, Am. J. Cancer **29**:47, 1937.

A significantly high correlation was found between the mitosis count in lymphosarcoma and the longevity and mortality from the tumor after biopsy. The study was objective in that the diagnosis was made by others, and the clinical outcome was not known to the author at the time the mitosis count was made. Cases were consecutive, and none were excluded which fulfilled the premises of the study. The mitosis count might prove to be a valuable aid in prognosis in the case of lymphosarcoma. A careful study of the deviations from the regression line may serve as a check on the efficacy of therapeutic procedure. Incidental observations on the duration of symptoms before biopsy, the age, the sex and the site of occurrence and a comparison of the mitosis counts in various diseases of the lymphatic system are recorded.

FROM THE AUTHOR'S SUMMARY.

TUMOR LIPIDS. F. L. HAVEN, Am. J. Cancer **29**:57, 1937.

The periphery of rat carcinosarcoma 256 consists of non-necrotic growing tissue; the center consists of necrotic nongrowing tissue. Analyses show that the average phospholipid content of the periphery is twice that of the center and that the center contains much more cholesterol ester and somewhat more free cholesterol, neutral fat and water than the periphery. The comparative lipid contents of the periphery and center lend support to the view that cholesterol ester and neutral fat are formed from fatty acids arising from decomposition of phospholipid—the fatty acids from two molecules of phospholipid esterifying one molecule of cholesterol and forming one molecule of neutral fat.

FROM THE AUTHOR'S SUMMARY.

GROWTH STIMULATING AND INHIBITING SUBSTANCES IN HUMAN URINE. G. L. ROHDENBURG and S. M. NAGY, Am. J. Cancer **29**:66, 1937.

Two substances, one accelerating the division rate of Colpidium campylum, the other inhibiting it, have been demonstrated in human urine. In normal persons and in those not having within the body a focus of proliferating cells the inhibiting agent dominates. In those having within the body a focus of proliferating cells the stimulating substances dominate.

FROM THE AUTHORS' CONCLUSIONS.

IDENTIFICATION OF TUMOR CELLS IN SEDIMENTS OF SEROUS EFFUSIONS. N. C. FOOT, Am. J. Path. **13**:1, 1937.

Zemansky's first criterion, the presence of fragments of tumor with the cells arranged in acini or papillae about a stroma that is definitely fibrovascular, stands uncontroverted. A nucleolar-nuclear ratio falling above 0.25 is of undoubted value; one of 0.30 or more, practically pathognomonic of the presence of tumor. Mesothelial pleural, pericardial and peritoneal covering cells present the chief obstacle in the way of successful diagnosis as they are readily confused with tumor cells on account of their large size and prominent nuclei. When they are measured, the dimensions are found to be quite uniform and regular; tumor cells, on the other hand, show a high nucleolar-nuclear ratio and a wide variation in measurements. Multinucleation is of no diagnostic value, and cell clumping is almost as worthless. Mitosis occurs in both positive and negative sediments, but monster, or abnormal, mitoses are found only in tumor cells. The occurrence of erythrocytes and fibrin is of little diagnostic value.

FROM THE AUTHOR'S SUMMARY.

CHLOROMA. E. V. KANDEL, Arch. Int. Med. **59**:691, 1937.

The literature on chloroma is reviewed from 1926 to the present, and three new cases are reported. The world literature contains reports of 175 indubitable cases of chloroma. With recent improvements in staining technic and better differ-

entiation of the acute lymphoid and myeloid leukemias, almost all the recent cases of chloroma have been reported as cases of myeloid leukemia, several standard texts to the contrary notwithstanding. The inevitable association of chloroma with myeloid leukemia should make it obvious that chloroma is simply a variant of myeloid leukemia, with the multipotential myeloblast assuming the distinctive role as type cell of an invasive neoplasm. FROM THE AUTHOR'S SUMMARY.

GONADOTROPIC HORMONE IN THE DIAGNOSIS OF CHORIONEPITHELIOMA. B. ZONDEK, J. A. M. A. **108**:607, 1937.

In the placenta affected by hydatidiform mole or by chorionepithelioma the gonadotropic hormone appears in greatly increased amounts in the blood, urine and spinal fluid. A diagnosis of hydatidiform mole cannot be considered as established unless in repeatedly performed examinations at least 200,000 mouse units of luteinizing principle is found in the urine and in addition a positive luteinizing reaction in the spinal fluid (preferably diluted). It is necessary to rule out toxemia of pregnancy, as in this condition large amounts of luteinizing substance are also excreted in the urine. However, in the latter case only a follicle-stimulating effect is obtained in the spinal fluid.

If the test for pregnancy still remains positive six weeks after the discharge of a hydatidiform mole and if the gonadotropic substance in the urine has progressively increased in this period, it suggests a diagnosis of chorionepithelioma, particularly if a positive reaction is also found in the spinal fluid. For confirmation of the diagnosis, exploratory curettage is necessary. If the results of the histologic examination are doubtful, the results of biologic assay of the urine and spinal fluid are of great significance.

In case the pregnancy test has become negative after the discharge of the hydatidiform mole and once more becomes positive within some time there is either a new pregnancy or a chorionepithelioma. The differential diagnosis must be established by clinical observation and by quantitative assays of the urine.

The assay of urine for gonadotropic substance is of importance for diagnosis and for prognosis in chorionepithelioma. If the pregnancy test has become negative following therapeutic measures, and if luteinizing substance occurs once more in the urine, this indicates that the malignant process is proceeding. Apparent clinical improvement may often be deceiving in such cases.

A considerable reduction in the amount of follicle-stimulating hormone excreted occurring suddenly without therapeutic measures is clinically a threatening sign, although when this occurs following therapy it is usually considered a favorable sign.

As the normal placenta is rich in gonadotropic material, the presence of 800 mouse units of luteinizing substance per gram of fresh tissue obtained by curettage must be established before one can diagnose a malignant condition. If 100 mouse units per gram is found in extra-uterine tumors, this indicates that the tumor is a metastasizing or extragenital chorionepithelioma.

Chorionepithelioma of the testicle or testicular teratoma with chorionepitheliomatous inclusions leads to a strongly increased production and discharge of follicle-stimulating and luteinizing factors. This increased excretion may also occur occasionally with other testicular tumors. It is absent in dysgerminoma. Tissue assay of testicular tumors furnishes valuable information as to the nature of the tissue. Dysgerminomas do not contain gonadotropic substance; the chorionepitheliomatous parts contain much of this factor.

The anterior pituitary in normal pregnancy, as well as in chorionepithelioma and in malignant testicular tumor, contains little or no gonadotropic hormone.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Nov. 20, 1937

ERNEST WITEBSKY, *President*

SAMUEL SANES, *Secretary*

PATHOGENICITY AND ANTIGENIC STRUCTURE OF SHIGELLA ALKALESCENS (ANDREWES). E. NETER and F. RAPPOLE.

Shigella alkalescens (Andrewes), which closely resembles the Flexner dysentery bacillus, is recorded to be nonpathogenic for man (Bergey, D. H.: Bergey's Manual of Determinative Bacteriology, ed. 4, Baltimore, Williams & Wilkins Company, 1934). Reports by Smith and Fraser, Popoff and Spanswick, Welch and Mickle and Weil, however, show that this micro-organism may cause diseases such as sepsis or infection of the urinary tract. On the other hand, its significance as a causative agent of intestinal disease is still under discussion. We recently observed three female patients suffering from acute infections of the urinary system which were caused by Shigella alkalescens. The assumption of the pathogenic significance of this micro-organism is supported by the facts that it was isolated on repeated occasions in pure culture and that two of the patients examined had specific antibodies against it.

S. alkalescens and the Flexner dysentery bacillus have certain similarities in antigenic structure: Flexner rabbit antiserums agglutinated four strains of S. alkalescens isolated in this laboratory (three from the urine of the patients mentioned and one from the stool of a normal person) and two strains of the American Type Culture Collection. Antiserums were prepared against four strains, each of which agglutinated all strains of S. alkalescens and also strains of the Flexner dysentery bacillus. It follows, first, that S. alkalescens has an antigen in common with the Flexner dysentery bacillus and, second, that our strains, as well as the two strains of the American Type Culture Collection, induce the formation of antibodies when injected into rabbits. Agglutinin absorption experiments revealed that Flexner and Hiss dysentery bacilli removed only the homologous antibodies from the S. alkalescens antiserum, while the absorbed antiserum still agglutinated all six strains of S. alkalescens. Thus, S. alkalescens contains two antigens, one in common with dysentery bacilli and a second which is different from those found in true dysentery bacilli and apparently characteristic of it.

A further attempt to analyze the antigenic structure of S. alkalescens was made in testing its susceptibility to dysentery bacteriophage. The phage was obtained through Dr. Philip Levine, of Newark, N. J.: Although this phage was found to act strongly on seven of eight strains of dysentery bacilli, it was found ineffective toward six strains of S. alkalescens, in spite of the fact that the Flexner and S. alkalescens strains were agglutinated by both the Flexner and the S. alkalescens antiserum. This result gives additional evidence of antigenic differences in both species.

INVAGINATION OF THE APPENDIX ASSOCIATED WITH CARCINOID TUMOR. ROBERT T. BOALS and JAMES C. SULLIVAN.

The following report is made for several reasons. The degree of invagination of the appendix in this case, i. e., an inversion of the entire appendix without participation of the cecal wall, represents the second rarest type. Further, the literature contains only one instance (Ansilloti) of appendical invagination asso-

ciated with carcinoid tumor. Clinically, the lesion in our case produced no symptoms; it was found incidentally at laparotomy for a fibroid uterus.

The case was as follows: A 41 year old housewife, white, married, complained of menorrhagia and metrorrhagia of three years' duration. On physical examination there was tenderness in the suprapubic region and left lower quadrant of the abdomen. At operation the uterus contained multiple fibroids. The left ovary showed multiple follicular cysts. The appendix could not be found at its normal site. A mass the size of a thumb, which arose from the junction of the longitudinal bands, was felt inside the cecum. The cecum was opened and the mass removed.

The growth consisted of a polyp-like structure, which weighed 14 Gm. and measured 4.2 by 2.4 by 2.2 cm., with a pedicle 1.3 cm. long and 1.2 cm. in diameter. This pedicle was a part of the appendix. The surface of the ovoid structure showed pinpoint-sized hemorrhages; its appearance was suggestive of mucosa. On section the structure was gray-yellow. The microscopic diagnosis was invagination of the appendix associated with a large carcinoid tumor involving all layers to the serosa.

PRIMARY LYMPHANGIOMA OF THE FALLOPIAN TUBE. S. SANES and ROBERT WARNER.

The pathologic observations in the eighteenth recorded case of primary lymphangioma of the fallopian tube are described.

An abdominal laparotomy was made on a 55 year old white married woman who had complained of a tumor in the abdomen and of vaginal bleeding for one and a half years. The corpus uteri, left tube and left ovary were removed.

The corpus was myomatous. It contained submucous and intramural fibroids ranging from cherry to lemon size. The endometrium was not hyperplastic. The left ovary measured 4 by 3 by 1 cm. and showed a corpus luteum with a central hemorrhage and a corpus haemorrhagicum. The left tube was 11 cm. in length. The fimbriated end was patent. At a point 8 cm. from the uterine attachment there was observed in the upper wall a moderately firm whitish nodule apparently well circumscribed and 1.1 cm. in diameter. The overlying peritoneum was stretched and glistening. The lumen was compressed. The right tube and ovary were noted at operation as being normal.

Microscopically the nodule noted grossly in the left fallopian tube was found to be situated between the tunica propria, muscularis and subserosa. Its structure was that of a meshwork of channels or spaces ranging from capillary to cavernous in size, with moderate stroma. The representative lining of the spaces consisted of flat endothelial cells. In the lumens were lymphocytes, desquamated endothelial cells and thin acidophilic fluid. In many spaces there was distinct evidence of endothelial proliferation, with cuboid, cylindric and polyhedral cells, stratification and giant cells. The cellular appearance of the stroma imitated the picture seen in angioblastic and reticulo-endothelial neoplasms. Fibers were chiefly of the reticulum and elastic type. In the periphery of the nodule were focal collections of round cells. The growth invaded the mucosa and muscularis. The histologic diagnosis was lymphangioma with distinct endothelial proliferation and with replacement of, and invasion into, tubal tissue.

A review of the literature disclosed that primary lymphangioma of the fallopian tube can be classified into two histologic groups: (1) simple lymphangioma and (2) lymphangioma with endothelial proliferation (lymphangioendothelioma?). Our case falls into the latter group. In this group the tumors were always small. They were discovered only as incidental findings in women operated on for, or dying from, other causes. The overlying peritoneum was not perforated. Metastases were never found. The degree of endothelial proliferation, destruction of tubal tissue and lack of encapsulation prompted some authors to make the histologic diagnosis of malignant growth. Other authors, despite the histologic appearances just mentioned, predicted a benign course for this type of lymphangioma of the fallopian tube.

PRIMARY TUBERCULOUS FOCI IN THE LUNGS WITHOUT FURTHER SPREAD TO LYMPH NODES IN HUMAN BEINGS. KORNEL TERPLAN and MARGUERITE T. LESLIE.

Several years ago two cases were presented before this society in which tuberculous foci in the lung were detected incidentally at postmortem examination of two children without corresponding lesions in the lymph nodes (Terplan, K., and Koenig, E. C.: ARCH. PATH. **16**:448, 1933). This presentation will add more evidence that first tuberculous infection of the lung may restrict itself to the primary parenchymal lesion, leaving no anatomic or histologic evidence of further spread from the primary focus to the regional lymph nodes. In all of these three cases the same technic was used: Roentgen photographs were taken of the lungs and tracheobronchial tree. In each case one small focus was found, giving a shadow of a calcified, or at least chalky, round lesion. All of these foci were histologically examined. In case 1, that of a 26 year old man, a typical cheesy pneumonic lesion was found, with a chalky center. In case 2, that of a 21 year old woman, the primary focus was of typical pneumonic structure with complete calcification and a thin bony wall. In the third case, which was that of a woman of 22 years, a minute calcified focus was found which showed histologically a typical bony ring around the calcified center.

In all three cases the regional lymph nodes draining the areas of the respective foci were examined in complete serial sections; the examination included all the bronchopulmonary, intrapulmonary and tracheobronchial lymph nodes. In no one of these cases were any changes found which have been interpreted as scars from tuberculosis, nor were there any active tuberculous lesions.

These findings point to a single Ghon focus, which is restricted to the lung and apparently healed. It is most probable that in such cases an exogenous reinfection may act like a first infection as far as the type of the focal lesion and spread to the regional lymph nodes are concerned. The cases presented appear so far the only completely controlled cases among very few similar investigations (Ghon; Ghon and Kudlich).

Book Reviews

The Harvey Lectures Delivered Under the Auspices of the Harvey Society of New York, 1936-1937. Under the patronage of the New York Academy of Medicine. by Dr. Wilder Penfield, and others. Series 32. Cloth. Price, $4. Pp. 245, with illustrations. Baltimore: Williams & Wilkins Company, 1937.

One notes the following lecturers and their subjects: Wilder Penfield on the cerebral cortex and consciousness; Eugene M. Landis on the passage of fluid through the capillary wall; S. Walker Ranson on some functions of the hypothalamus; R. Schoenheimer on investigation of intermediary metabolism with the aid of heavy hydrogen; Thorvald Madsen on the scientific work of the health organization of the League of Nations; Herbert S. Gasser on the control of excitation in the nervous system; C. N. H. Long on the influence of the pituitary and adrenal glands on pancreatic diabetes; Sir Henry Dale on transmission of nervous effects by acetylcholine. The lectures summarize well the recent advances of investigation on the problems represented.

Books Received

DISEASES OF THE BLOOD AND ATLAS OF HEMATOLOGY WITH CLINICAL AND HEMATOLOGIC DESCRIPTIONS OF THE BLOOD DISEASES INCLUDING A SECTION ON TECHNIC AND TERMINOLOGY. Roy R. Kracke, M.D., Professor of Bacteriology, Pathology and Laboratory Diagnosis, Emory University School of Medicine, Atlanta, Ga., and Hortense Elton Garver, M.S., Instructor in Laboratory Diagnosis, Emory University School of Medicine. Cloth. Price, $15. Pp. 532, with 61 illustrations. Philadelphia: J. B. Lippincott Company, 1937.

PHENOMENON OF LOCAL TISSUE REACTIVITY AND ITS IMMUNOLOGICAL PATHOLOGICAL AND CLINICAL SIGNIFICANCE. Gregory Shwartzman, M.D., Bacteriologist, the Mount Sinai Hospital, New York. Foreword by Jules Bordet, M.D., Paris. Cloth. Price, $7.50. Pp. 461, with 68 illustrations. New York: Paul B. Hoeber, Inc., 1937.

A MONOGRAPH ON VEINS. Kenneth J. Franklin, D.M., M.R.C.P., Tutor and Lecturer in Physiology, Oriel College; University Demonstrator of Pharmacology and Assistant Director of the Nuffield Institute for Medical Research, Oxford. Cloth. Price, $6. Pp. 410, with 46 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

LEÇONS CLINIQUES SUR LES AFFECTIONS HYPOPHYSAIRES. L. Langeron, professeur de clinique médicale à la Faculté libre de Lille. Suivi d'un chapitre sur la physiologie des affections hypophysaires par R. Desplats, professeur d'electroradiologie clinique à la Faculté libre de Lille. Paper. Price, 50 francs. Pp. 222. Paris: Masson & Cie, 1937.